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ORIGINAL RESEARCH PAPER

A PRELIMINARY STUDY ON USING ULTRASOUNDS FOR THE VALORIZATION OF SPENT BREWER'S YEAST

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Abstract

Spent brewer's yeast is a by-product rich in components with biological activity, being considered an innovative and economical viable solution for creating sustainable functional food systems. This work describes the use of ultrasound treatment, performed under various conditions, with the aim to facilitate the cell lysis and to further characterize the released cellular content of the spent brewer's yeast. Solid residue content of the spent yeast suspensions was positively influenced by both the amplitude and the exposure time ($p<0.05$), reaching a maximum content (40.02±0.13 g/100 g dry yeast) at 70% amplitude, after 7.5 minutes of ultrasound treatment under pulsation mode, and the minimum after 2.5 minutes of exposure at 20% ultrasound amplitude under continuous mode (31.59±1.2 g/100 g dry yeast). The content of the soluble proteins released from the yeast cells during the ultrasound treatment under pulsation mode, reached a maximum increase at amplitude of 70%, after 7.5 minutes, being by 85% higher compared to the control represented by untreated yeast suspension. The disintegration index, *Z,* indicated that the maximum degree of yeast cells disintegration was obtained when applying the ultrasound treatment under pulsation mode for 5 minutes, at 50% amplitude. The antioxidant activity of the yeast extracts depended on the ultrasound conditions applied, the maximum antioxidant activity being measured in the sample exposed to ultrasound under pulsation mode for 5 minutes at 50% amplitude. In conclusion, ultrasound is a good approach to release the intracellular components from the spent brewer's yeast. Further studies factoring ultrasounds power and frequency should be conducted, such to identify the optimum set of parameters which allow the highest release of the intracellular yeast compounds.

Keywords: spent brewer's yeast, bioactive ingredients, ultrasound, protein, antioxidant activity

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Introduction

Nowadays, food industry faces important challenges due to the changes in the consumers interests, who do not longer demand for food that satisfies the nutritional and sensorial needs, but go beyond, asking for food with health enhancing benefits that control and prevent diseases (Sharma, 2019). On the other hand, food industry produces large quantities of by-products and residues. A recent EU project showed that more than 30 million tons of food waste is generated annually by the food processing sector (Moates *et al.*, 2016). An important challenge raises up, consisting on the regeneration, reuse of these by-products and residues and innovation to make the transition towards circular economy, while satisfying consumers demands for healthy foods (Maqbool *et al.*, 2020; Olivares-Galvan *et al*., 2022).

Brewing industry generates large amounts of agro-industrial residues, such as spent grain, yeast and trub that, despite their nutritional potential and availability throughout the year, are under-used as animal feed and considered an organic waste (Marson *et al*., 2020a). Therefore, it is essential to find solutions for recovery and valorization of such by-products (Moates *et al*., 2016). Spent brewer's yeast (SBY), the second largest by-product of the brewing industry, is a thick and viscous suspension with a solid concentration between 3-15% (w/v). SBY are a GRAS (generally recognized as safe) source of protein (45-60%), rich in essential amino acids, with high potential to be used as dietary supplements (Thakkar *et al*., 2021). Similar to other food proteins, because of their functional properties in terms of foaming, emulsifying, antioxidant, antimicrobial and encapsulating properties, SBY and yeast proteins have a good potential for the development of novel functional food ingredients/products (San Martin *et al.*, 2021; Thaís *et al.*, 2020). The first step in processing the SBY biomass is to disrupt the yeast cell walls such as to enable the access to cell debris and intracellular compounds, followed by the separation of the released targeted compounds from the remaining cell components (Oliveira *et al*., 2022; Marson *et al*., 2020b). The methods applied for the disruption of the yeast cells are grouped into mechanical and non-mechanical methods, and play an important role in defining the composition of the yeast extract obtained after the disruption of the yeast cell wall. Mechanical methods are based on the disruption, without micronisation of the cell debris for releasing profiles of the targeted compounds, being frequently applied at the industrial scale (Jaeger *et al*., 2020; Liu *et al*., 2016). Ultrasound treatment is a mechanical method commonly applied for cell disruption and extraction of intracellular components. Ultrasonication creates high shear forces by high-frequency ultrasound, finally resulting in high level disintegration of the cell wall. Ultrasonication is widely used to recover proteins from microbial cells. The recovery of protein by using ultrasound is independent on the cell concentration and correlated to the acoustic power (Liu *et al*., 2013).

The aim of the present study was to investigate the effect of different ultrasonic variables, like amplitude, pulsation mode and holding time, on the SBY cells disruption and the release of soluble proteins.

Materials and methods

Materials

The inactivated dried SBY (*Saccharomyces cerevisiae*) was kindly provided by a beer factory from Ploiesti, Romania. The powder was stored under refrigeration until use. The crude protein content determined according to the Dumas method by using a conversion factor of 5.5 was 36.85±0.23 g/100 g dried sample. This conversion factor is specific to yeast proteins as it contains high levels of non-proteic nitrogen. 1,1-diphenyl-2-pycryl hydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

Preparation of yeast suspension

Yeast suspensions at concentration of 10 % (w/v) were prepared by mixing the SBY with distilled water, followed by stirring for 15 minutes at 100 rpm for complete hydration.

Ultrasonication process

The ultrasonication treatments were performed using an ultrasonic processor (Sonopuls HD 3100, Bandelin electronic, Berlin, Germany) equipped with a 13-mm sonotrode probe made of titanium (VS 70 T). The yeast suspension samples were processed using the following parameters: sonication mode either continuous or pulsation (5 sec. on, 5 sec. off), amplitude from 20% to 70%, while sonication time from 2.5 to 7.5 minutes. The frequency and power were maintained constant at 20 kHz and 200 W, respectively. During processing, the US probe was submerged to a depth of 50 mm in a 50 mL Falcon tube containing 25 mL of sample. The temperature was maintained constant during the ultrasound treatments, by immersing the Falcon tubes into a beaker containing ice.

Obtaining the SBY extract

Upon ultrasonication, the yeast suspensions were centrifuged for 20 minutes at 9000 rpm and 4°C, the resulting extract being further used for determination of solid residues, soluble protein content, fluorescence and UV-VIS measurements, as well as for the evaluation of antioxidant activity. The extract of the SBY with no ultrasonication treatment was used as control.

Yeast cells disintegration

The electrical conductivity of the ultrasonicated suspensions was measured at room temperature using a conductivity meter. The electrical conductivity of the suspensions was associated to the extraction of ionic intracellular components resulted from the damaged cells exposed to ultrasounds (Liu *et al*., 2013). The damage of the yeast cells was evaluated using the conductivity disintegration index (Z) as follows:

$$Z = (\sigma - \sigma_i)/(\sigma_{max}-\sigma_i) \qquad (1)$$

where σ is the electrical conductivity of the yeast suspension after the ultrasound treatment, σ_i is the initial electrical conductivity of the yeast without the ultrasound treatment, whereas σ_{max} is electrical conductivity of the yeast suspension at maximum disintegration of the cells. Based on the equation (1), *Z* score ranges from 0 (for untreated yeast cells) to 1 (cells exposed to maximum disintegration). The maximum disintegration of the yeast cells was obtained after 30 minutes of ultrasound treatment at 200 W power and 70 % duty cycle.

Solid residue content

The solid residue content (SRC) was determined as follows: 1 mL of yeast extract was transferred to glass vials and dried for 24 h in an oven set at 105 ± 2 °C. After drying, the gross weight of the vials and samples was measured, the solid residue being expressed as g per 100 g of dry SBY.

Soluble proteins content

The soluble proteins content released from the yeast cells after the ultrasound treatments was evaluated in the yeast extract according to the Lowry method (Lowry *et al*., 1951).

Antioxidant activity

The antioxidant activity was evaluated using the $ABTS^{\cdot+}$ radical reaction assay and the DPPH radical scavenging activity.

Fresh $ABTS^{\cdot+}$ solution was prepared as described previously by Mihalcea *et al*. (2021). A volume of 20 µL of yeast extract was mixed with 1.98 mL of $ABTS^{\cdot+}$ solution and allowed to react for 20 minutes in the dark. The absorbance of the solution was monitored at 734 nm.

Regarding the DPPH radical scavenging activity assay, a volume of 50 µL of yeast extract was mixed with 1.95 mL of fresh DPPH solution (3 mg of DPPH in 100 mL methanol). The reaction mixture was kept for 20 minutes under dark at room temperature, and the absorbance was afterwards recorded at 515 nm.

The antioxidant activity was expressed as inhibition rate (%) using the equation 2:

$$\text{Inhibition (\%)} = (1 - A/A_0) \cdot 100 \qquad (2)$$

where A is the absorbance of the sample and A_0 is the absorbance of control sample prepared with methanol 80%, instead of yeast extract.

Fluorescence spectroscopy analysis

For the fluorescence measurements, a volume of 0.1 mL of yeast extract was dispersed in 3.0 mL Tris buffer (3 mM, 7.5 pH) to measure tryptophan (Trp) and tyrosine (Tyr) fluorescence intensity, upon excitation at wavelengths of 295 nm and 274 nm, respectively. Fluorescence emission spectra were collected between 310-420 nm using a LS-55 Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). The width of the excitation and emission slits was set at 10 nm.

UV-VIS measurements

The UV absorption spectrum of the yeast supernatant was recorded by a UV-VIS Cintra 202 spectrophotometer (GBC Scientific Equipment, Braeside, Australia). A volume of 50 µL of sample was mixed with 3 mL of distilled water and the absorbance was measured between 200–400 nm in a quartz cuvette with a 10 mm path length.

Statistical analysis

The results are expressed as mean values followed by standard deviation. The differences between samples were evaluated by one-way ANOVA. Posthoc test via Tukey method was performed when p value in ANOVA method was less than 0.05.

Results and discussion

Effect of ultrasound treatment on cells disintegration

SBY cell walls contain 10-15% proteins and about 85-90% polysaccharides. On the other hand, proteins are the most predominant fraction within the yeast cells, thus the release of proteins represents a good indicator to evaluate the physical disruption of the cell membrane (Wu *et al.*, 2015). In addition to the protein content, the degree of disintegration of the yeast cells can be evaluated by using the disintegration index Z. In this study, the effect of the ultrasonication amplitude (ranging between 20 and 70%) and time (ranging between 2.5 and 7.5 minutes) was investigated. The Z values determined for the eighteen samples obtained through ultrasound processing under different conditions are presented in Table 1. When ultrasound treatment was performed under continuous mode (Table 1), at 20% amplitude, the Z values increased by increasing the holding time from 0.54±0.05 after 2.5 minutes of ultrasound treatment to 0.80±0.10 after 7.5 minutes. At 50% and 70% amplitude, under continuous mode of ultrasound treatment and longer holding times, the Z values were lower compared to those measured at 20% amplitude, indicating that increasing amplitude and holding time applied in this study, did not exert a positive effect on the disintegration of SBY cells. Regarding the ultrasound treatment performed using pulsation mode, from Table 1 it can be seen that at 20% amplitude, Z values increased with increasing the holding time of ultrasound. At 50% amplitude, the highest Z value was obtained after 5 minutes of ultrasound treatment (0.90±0.05), indicating the maximum degree of disintegration for SBY cells. Further increase of the ultrasound treatment up to 7.5 minutes caused the decrease of the Z value to 0.76±0.03. At 70% amplitude, Z values decreased with increasing the holding time, suggesting that higher disintegration of SBY cells can be achieved when higher amplitude values are applied for a short period of time.

Performing the ultrasound treatment under continuous mode, at lower amplitude and higher holding time generated similar disintegration degree of the SBY cells. However, under pulsation mode, the exposure of the SBY cells to higher amplitude for longer time, disintegrated the SBY cells to a lesser extent. Liu *et al*. (2013)

investigated the disruption of *Saccharomyces cerevisiae* cells and protein release kinetics by using a horn-type sonicator (120 to 600 W and 20 kHz) and ultrasonic bath (25 kHz, 600 W). The authors reported that the effect of horn type sonication was stronger than ultrasonic bath for cell disruption and protein release, the highest disintegration index being obtained at 600 W and 50% duty cycle. In other studies, the effect of high-pressure treatment and pulsed electric fields on the disintegration of yeast proteins was investigated, and the disruption of yeast cells evaluated in terms of disintegration index *Z* was positively correlated with the pressure and the pulsed electric fields (Dimopoulos *et al.*, 2018, Dimopoulos *et al.*, 2021).

Effect of ultrasound treatment on the release of solid residue from yeast cells

The SRC of the control sample was 29.86±1.2 g/100 g dry yeast, whereas, the SRC of the yeast extracts exposed to ultrasound treatment resulted after centrifugation is presented in Table 1. It can be seen that when the ultrasound treatment was applied without pulsation, the SRC of the SBY suspensions was positively influenced by both the amplitude and the exposure time ($p<0.05$). For example, when the ultrasound treatment was performed at 20% and 70% amplitude, the SRC increased by increasing the time from 2.5 minutes to 7.5 minutes by about 10% and 6.3%, respectively. The lowest SRC was measured after 2.5 minute of exposure at 20% ultrasound amplitude (31.59±1.2 g/100 g dry yeast) and the highest at 70% after 7.5 minutes of ultrasound treatment (37.13±0.1 g/100 g dry yeast). When the ultrasound treatment was performed under pulsation mode, the applied amplitude exerted a significant effect on the SRC of the SBY extracts. For example, applying an ultrasound treatment for 2.5 minutes (compared to the control sample) increased the SRC by 8.3% at 20% amplitude to 20% at 70 % amplitude, whereas after 7.5 minutes of ultrasound treatment, the SRC showed the highest value of all tested samples (40.02±0.13 g/100 g dry yeast). Based on the above-mentioned results, in order to obtain higher SRC, ultrasound treatment should be performed under pulsation at high amplitude and holding times.

Effect of ultrasound treatment on the release of soluble proteins from yeast cells

Proteins are the main remaining cell component separated during the yeast cell lysis. Thus, waste streams from the extraction processes are rich in soluble protein that can be processed to recover proteins and peptides in a circular economy-based approach (Oliveira *et al*., 2022). The soluble protein content (SPC) in the untreated sample (control) was 19.1±0.9 g/100 g of protein dry SBY. The SPC released from the SBY cells during the ultrasound treatment are presented in Table 1. The SPC increased during the ultrasound treatment and was influenced by amplitude, pulsation mode and holding time ($p<0.05$). Compared to the untreated sample, at 20% amplitude and no pulsation, after 5 minutes of ultrasound treatment, SPC increased by about 25% ($p<0.05$), whereas increasing the holding time to 7.5 minutes did not favor the release of the intracellular content of SPY cells, as SPC did not show significant differences (Table 1). Moreover, increasing the amplitude to 70% resulted in the highest increase of the SPC, released by SBY after 7.5 minutes of ultrasound exposure (by about 53% compared to the untreated sample;

$p<0.01$). The SPC of the sample obtained through ultrasound treatment under these conditions was 29.4±1.2 g/100 g protein dry yeast.

With few exceptions, applying the ultrasound treatment under pulsation mode generated lower SPC values compared to those resulted without pulsation. Jacob *et al.* (2019a) studied several cell disruption methods to produce yeast extracts with increased protein content and showed that after 10 minutes of exposure to ultrasound, the effectiveness based on the nitrogen extraction yield was about 20%, the highest effectiveness of about 80% being obtained after 2 hours of ultrasound treatment at 400 W without pulsation. In our study, the SPC content varied from 8.20±0.46 to 10.85±0.46 g/100 g dry yeast under ultrasound treatment without pulsation and from 7.29±1.05 to 13.58±1.75 g/100 g dry yeast under pulsation mode. The results reported in this study are different compared to the study performed by Jacob *et al.* (2019b). The authors reported the composition of the yeast extracts produced by using cell mil, sonotrode and autolysis and regarding the protein content, they measured in the yeast extracts exposed to ultrasound for 30 minutes at 400 W a protein content of 492.22±9.21 mg/g dry weight. Our results are in agreement with those reported by García Martín *et al.* (2013), who indicated that the application of ultrasound treatment on the light lees resulted in an increase of the protein concentration.

Antioxidant activity

The DPPH and $ABTS^{\cdot+}$ radical-scavenging values are presented in Table 1. In the samples exposed to ultrasound in the continuous mode, when the antioxidant activity was assessed by using DPPH, the inhibition started from 23.44±0.20 % in the control sample and reached a maximum of 25.33±0.13 % at 50% amplitude after 5 minute of ultrasound treatment. The highest antioxidant activity (41.35±0.13 %) was calculated when the ultrasound was performed under pulsation mode at 50% amplitude, after 2.5 minutes of ultrasound. The inhibition rate values evaluated by $ABTS^{\cdot+}$ method were significantly ($p<0.05$) higher than those measured by DPPH method. This difference might be associated to the various solubility and diffusivity of the free radicals in the reaction medium (Mirzaei *et al*., 2015). The results of our study are similar with those reported in the literature (Mirzaei *et al.,* 2015; Nalinanon *et al.,* 2011). The high reactivity of the $ABTS^{\cdot+}$ is seen as being advantageous as it reacts with many antioxidants. However, as $ABTS^{\cdot+}$ is highly instable, it is recommended to sustain the results with another scavenging activity method like DPPH (Oliveira *et al.,* 2022).

Fluorescence spectroscopy analysis

Fluorescence spectroscopy measurements are frequently applied to evaluate the changes in Trp and Tyr surroundings of proteins, and a useful indicator to assess protein conformation and the loss of amino-acids (Zhang *et al*., 2014). Fluorescence characteristics of Trp and Tyr residues of the SBY proteins expressed in terms of maximum intensity (I_{max}) and maximum emission wavelength (λ_{max}) obtained after applying an ultrasound treatment in various conditions, are presented in Table 2.

Table 1. Composition and antioxidant activity of the extracts obtained from spent brewer's yeast exposed to ultrasound treatment.

Amplitude, %	Time, min	Pulsation mode	Disintegration index	SRC, g/100 g dry yeast	SPC, g/100 g dry yeast	DPPH Inhibition rate%	$ABTS^{\cdot+}$ Inhibition rate%
20	2.5	Off	0.54 ± 0.05^{bC}	31.59 ± 1.2^{aB}	8.20 ± 0.46^{aC}	23.98 ± 0.13^{bB}	62.04 ± 0.09^{bC}
20	5	Off	0.60 ± 0.06^{bB}	33.02 ± 1.34^{aB}	8.63 ± 0.60^{abC}	24.46 ± 0.13^{aB}	67.53 ± 0.09^{aA}
20	7.5	Off	0.80 ± 0.10^{aA}	34.67 ± 0.40^{aC}	8.53 ± 0.23^{aC}	22.37 ± 0.75^{bB}	62.18 ± 0.09^{bC}
50	2.5	Off	0.61 ± 0.02^{bA}	33.92 ± 0.03^{bA}	8.70 ± 0.70^{bB}	22.96 ± 0.06^{bC}	67.46 ± 0.19^{bA}
50	5	Off	0.61 ± 0.01^{bA}	34.17 ± 1.27^{bB}	9.28 ± 0.81^{aB}	25.33 ± 0.13^{aA}	67.88 ± 0.19^{aA}
50	7.5	Off	0.67 ± 0.03^{aB}	36.08 ± 0.40^{aB}	8.87 ± 0.70^{bB}	20.48 ± 0.68^{cC}	67.04 ± 0.19^{bB}
70	2.5	Off	0.60 ± 0.04^{bB}	34.83 ± 0.14^{bA}	9.61 ± 1.52^{cA}	21.70 ± 0.20^{abA}	61.54 ± 0.20^{bB}
70	5	Off	0.61 ± 0.03^{bA}	37.12 ± 0.01^{aA}	9.94 ± 0.11^{bA}	24.22 ± 0.61^{aB}	67.81 ± 0.29^{aA}
70	7.5	Off	0.65 ± 0.05^{aC}	37.13 ± 0.10^{aA}	10.85 ± 0.46^{aA}	23.68 ± 0.13^{bA}	67.81 ± 0.09^{aA}
20	2.5	On	0.60 ± 0.05^{cB}	36.09 ± 3.79^{aB}	7.29 ± 1.05^{cB}	19.66 ± 0.13^{bC}	67.81 ± 0.09^{aB}
20	5	On	0.72 ± 0.10^{bB}	33.1 ± 0.02^{aC}	8.87 ± 1.63^{bB}	19.41 ± 0.13^{bC}	65.70 ± 0.29^{bC}
20	7.5	On	0.80 ± 0.10^{aA}	34.8 ± 0.76^{aC}	10.36 ± 3.04^{aA}	22.59 ± 0.75^{aB}	64.15 ± 0.09^{cB}
50	2.5	On	0.60 ± 0.08^{cB}	32.98 ± 2.01^{bB}	7.13 ± 0.11^{bB}	24.61 ± 0.06^{cB}	66.19 ± 0.19^{cC}
50	5	On	0.90 ± 0.05^{aA}	37.2 ± 0.11^{aB}	8.29 ± 1.05^{aB}	41.35 ± 0.13^{aA}	86.97 ± 2.09^{aA}
50	7.5	On	0.76 ± 0.03^{bA}	37.56 ± 0.32^{aB}	6.96 ± 0.58^{cC}	27.37 ± 0.68^{bB}	79.43 ± 3.58^{bA}
70	2.5	On	0.78 ± 0.08^{aA}	37.17 ± 0.48^{cA}	8.29 ± 0.81^{cA}	26.11 ± 0.20^{bA}	70.84 ± 4.32^{bA}
70	5	On	0.58 ± 0.07^{bC}	38.72 ± 0.66^{bA}	13.58 ± 1.75^{aA}	20.43 ± 0.61^{cB}	68.87 ± 0.39^{cB}
70	7.5	On	0.54 ± 0.05^{cB}	40.02 ± 0.13^{aA}	8.87 ± 0.70^{bB}	29.7 ± 0.13^{aA}	79.29 ± 0.79^{aA}

Mean values that for the same amplitude and column do not share the same letter (a, b, c) are statistically different at $p < 0.05$, based on posthoc Tukey method.

Mean values that for the same time and column do not share the same letter (A, B, C) are statistically different at $p < 0.05$, based on posthoc Tukey method.

Regarding the fluorescence of the Trp (Table 2), it can be seen that when ultrasound treatment was applied without pulsation at 20% amplitude, the increase of holding time increased Trp fluorescence from 127.9±2.1 a.u. to 134.1±0.5 a.u. On the other hand, at higher amplitude values, regardless of the holding time, the Trp fluorescence remained constant. The fluorescence intensity of the Tyr residues was not affected by the ultrasound treatment conditions performed under continuous mode. On the other hand, by applying the ultrasound treatment under the pulsation mode, the maximum fluorescence intensity decreased by about 20% at 50% amplitude and 7.5 minutes of ultrasound treatment.

Table 2. The maximum fluorescence intensity (FI_{max}) and maximum emission wavelength (λ_{max}) of Trp and Tyr residues originating from the proteins and peptides of SBY treated with ultrasound without pulsation (a) and pulsation (b).

Amplitude %	Time, min	Pulsation mode	Trp		Tyr	
			λ_{max}	FI max	λ_{max}	FI max
0	**0**	**Off**	365.0	124.7±2.4^{a}	361.5	60.4±1.4^{a}
20	**2.5**	**Off**	364.0	127.9±2.1^{a}	360.0	61.2±1.6^{a}
20	**5**	**Off**	363.5	129.4±3.9^{a}	361.0	57.9±2.8^{a}
20	**7.5**	**Off**	363.5	134.1±0.5^{a}	360.5	61.3±2.4^{a}
50	**2.5**	**Off**	363.5	125.4±1.9^{b}	361.0	57.5±3.0^{a}
50	**5**	**Off**	363.5	126.9±1.2^{b}	360.0	61.0±2.1^{a}
50	**7.5**	**Off**	363.0	126.2±1.9^{a}	360.0	57.4±2.4^{a}
70	**2.5**	**Off**	363.0	125.2±1.1^{a}	360.0	56.0±1.9^{a}
70	**5**	**Off**	362.5	127.6±3.7^{a}	361.0	55.3±1.2^{a}
70	**7.5**	**Off**	362.5	126.9±1.2^{a}	360.0	58.9±1.1^{a}
0	**0**	**On**	365.0	124.7±2.4^{a}	361.5	60.4±1.4^{a}
20	**2.5**	**On**	364.5	125.8±1.8^{a}	360.5	57.6±0.5^{c}
20	**5**	**On**	364.5	123.0±2.9^{a}	360.5	56.3±0.8^{c}
20	**7.5**	**On**	363.5	124.1±2.5^{a}	360.5	57.9±1.2^{b}
50	**2.5**	**On**	363.5	127.6±1.2^{a}	360.0	53.8±1.1^{a}
50	**5**	**On**	364.5	129.2±1.2^{a}	359.5	55.4±0.8^{a}
50	**7.5**	**On**	363.0	131.9±1.5^{a}	360.5	49.0±2.4^{b}
70	**2.5**	**On**	364.0	128.9±1.3^{b}	360.5	56.5±3.1^{a}
70	**5**	**On**	362.5	131.0±1.7^{b}	359.5	50.8±0.8^{c}
70	**7.5**	**On**	363.0	134.6±2.2^{a}	359.5	51.1±0.9^{b}

Mean values that for the same amplitude and column do not share the same letter (a, b, c) are statistically different at $p < 0.05$, based on posthoc Tukey method.

The pulsation mode, amplitude and holding time during the ultrasound treatment influenced the λ_{max} registered in case of the measurements at both excitation wavelengths, set to collect information on Trp and Tyr fluorescence. Analyzing the

results showing details on the Trp residues, presented in Table 2, one can see that increasing the holding time decreased the maximum emission from 365 nm, corresponding to the control sample, to 362.5 nm after 7.5 minutes of ultrasound treatment at 70% under continuous mode, and by 2 nm under pulsation mode. For Tyr residues, a blue shift of only 1.5 nm was measured for the ultrasound treated sample in the continuous mode, and of 2 nm under pulsation mode (Table 2). These blue shifts indicate that both Trp and Tyr residues get a higher exposure to a more nonpolar microenvironment when subjected to the ultrasound treatment. The fluorescence intensity associated to the Trp residues was always higher compared to the one associated to the Tyr residues, indicating the higher overall exposure of the Trp residues originating from the intracellular yeast proteins and peptides.

UV-VIS measurements

The extraction efficiency of the high molecular weight compounds was evaluated by using UV-VIS analysis, the absorbance spectra of the supernatant solutions of the SBY being presented in Figure 1.

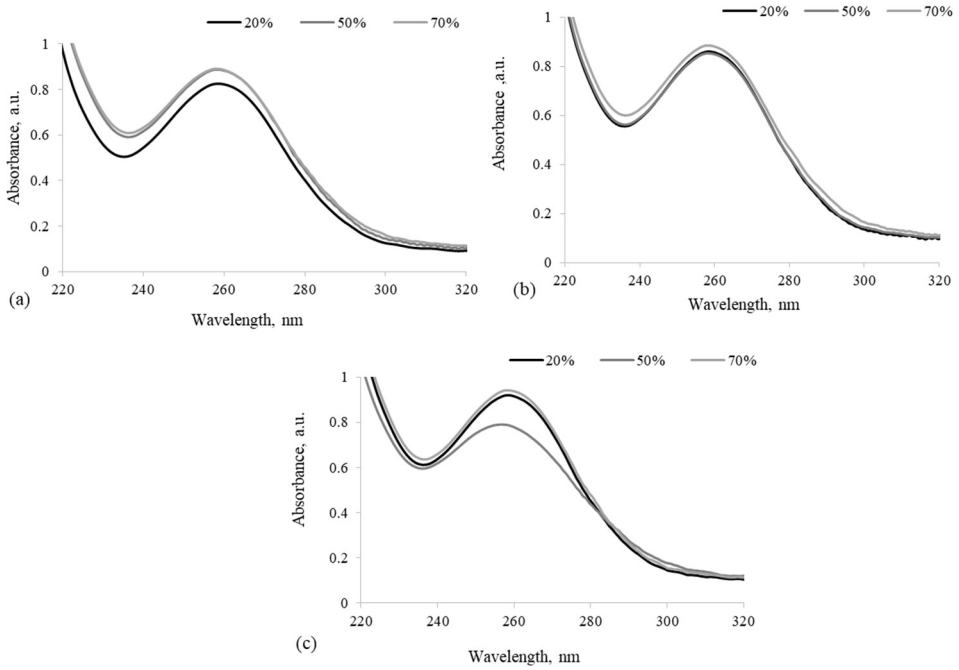

Figure 1. Effect of sonication under continuous mode on the absorbance of SBY extract. The sonication was performed for 2.5 min (a) 5.0 min (b) and 7.5 min (c) at different amplitudes (20, 50 and 70%).

The obtained peak indicates the presence of proteins in the samples, one of the main intracellular constituents of *S. cerevisiae* (Liu *et al*., 2013). When ultrasound was performed under continuous mode, at 20% amplitude, the absorbance

increased with increasing the holding time, thus indicating better release of the intracellular compounds. A similar trend was registered in case of the samples treated at 70% amplitude, the highest absorbance being measured after 7.5 minutes of ultrasound treatment. On the other hand, the decrease of the absorbance of the samples with the increase of the holding time was observed when the ultrasound treatment was carried out at amplitude of 50%.

The absorbance measured for the samples ultrasonicated under pulsation mode (Figure 2) did not show significant differences compared to the samples exposed to continuous ultrasounds.

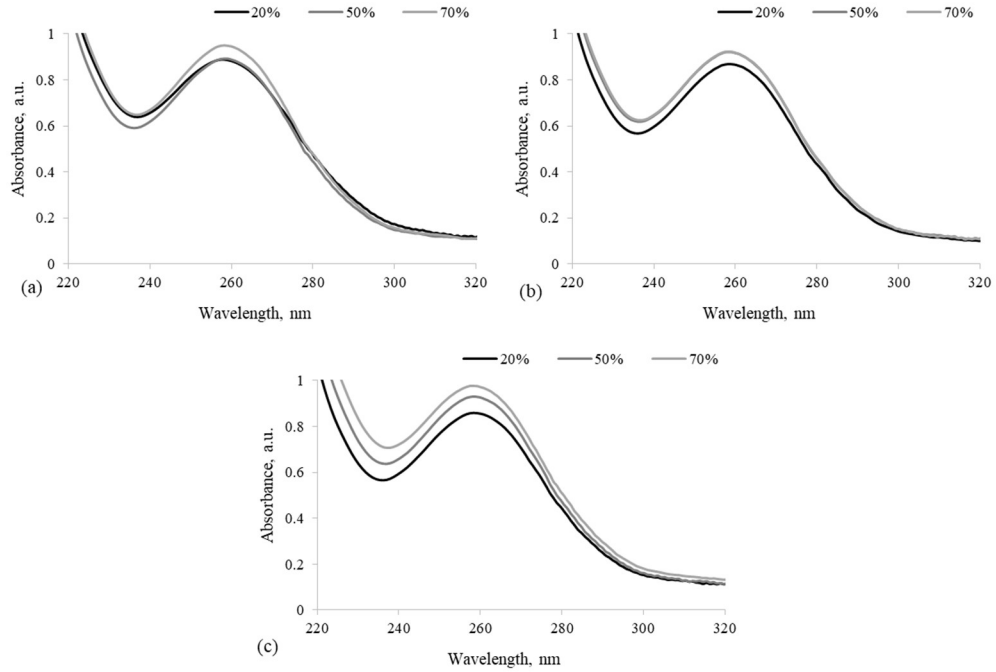

Figure 2. Effect of sonication under pulsation mode on the absorbance of SBY extract. The sonication was performed for 2.5 min (a) 5.0 min (b) and 7.5 min (c) at different amplitudes (20, 50 and 70%).

Conclusions

Spent brewer's yeast was treated by ultrasound treatment, under continuous and pulsation mode at amplitudes ranging from 20% to 70% for 2.5, 5 and 7.5 minutes with the aim to disrupt the yeast cells and to improve the release of the intracellular components. The efficiency of the treatment was evaluated in terms of solid residue content, soluble protein content, disintegration index and antioxidant activity of the extracts. The highest solid residue content was reached using ultrasound under pulsation mode at 70% for 7.5 minutes. On the other hand, the ultrasound applied under continuous mode favored the release of more soluble

proteins than under the pulsation mode. Ultrasonication for 5 minutes at 50% amplitude under pulsation mode, resulted in the maximum disruption of the spent brewer yeast cells and highest antioxidant activity. Taking into account the above-mentioned results, the yeast extract with the highest soluble content and antioxidant activity will be evaluated for obtaining peptides with increased functionality.

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