SCIENTIFIC REPORT

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EMERGING STRATEGIES TO IMPROVE THE POTENTIAL OF SPENT BREWER YEAST PROTEINS FOR CREATING NEW FUNCTIONAL FOODS

(Acronym: FunYeast)

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PHASE 1

OBJECTIVE: To test the susceptibility to lysis of different spent brewer yeast repitched or non repitched such as to disrupt the cell wall and release the proteins and peptides, whose functionality will be tailored using ultrasound/high pressure.	**Degree of achievement 100%**

1. Exploring SBY to produce peptides/protein hydrolysates

Activity 1.1. Pre-treatment of spent brewer yeast (SBY) using several yeast lysis procedures such as mechanical/ plasmolysis/ autolysis/enzymatic hydrolysis to break down the cell walls to release the yeast proteins;

This activity was performed using wet spent yeast from the Brewing Pilot Plant of the Faculty of Food Science and Engineering, Galati and dry SBY donated by an industrial beer processor from Ploiesti. The spent yeast was characterized regarding protein content and dry matter content.

Table 1. Protein and dry matter content

Yeast type	Protein content g/100 g sample	Dry matter, g/100 g sample
Wet yeast	13.50±0.35	26.84±0.78
Dry yeast	36.85±0.23	88.41±0.81

The lysis procedures tested for the disruption of cell wall and release of proteins were:

Autolysis: yeast cells were mixed with distilled water at a concentration of 15% (w/v), incubated under shaking at 100 rpm at 55°C, for 48 h. After that, the suspension was inactivated at 80°C, for 10 min, cooled and centrifuged, the resulting supernatant had a protein content of 2.24 g/100 g dry yeast, dry matter content of 7.58% and inhibition rate of 74.10 ± 7.46 %.

Lysis assisted by high pressure: frozen wet SBY was tawed at room temperature and 3 g of biomass (20%) was subjected to several combinations of temperature and pressure using a high-pressure equipment available at the Bioaliment TehnIA Research Center from the Faculty of Food Science and Engineering as follows:

- 300 MPa, for 0, 5, 10, 15,20, 25, 30 minutes;
- 400 MPa, for 0, 5, 10, 15,20, 25, 30 minutes;
- 500 MPa, for 0, 5, 15,20, 25, 30, minutes;

The supernatant was analysed in terms of soluble protein content and antioxidant activity (Table 2).

Table 2. The soluble protein content and antioxidant activity of high-pressure treated yeast samples

Pressure MPa	Holding time (minutes)						
	0	5	10	15	20	25	30
Soluble proteins (% wet yeast)							
300	13,98±0,5	13,20±1,02	13,05±1,61	13,84±3,96	15,78±0,82	10,71±1,65	15,38±0,27
400	5,47±0,25	9,89±0,05	8,75±0,0	10,61±0,01	9,53±0,01	10,18±0,01	9,13±0,02
500	11,04±0,89	9,41±0,04	-	9,30±0,43	10,07±0,31	9,30±0,43	9,80±0,16
Antioxidant activity, DPPH Inhibition rate (%)							
300	81,78±0,0	78,81±0,07	64,39±0,0	74,45±0,14	81,57±0,0	71,36±0,07	76,02±0,00
400	66,53±0,47	66,53±0,47	56,33±4,96	66,20±0,63	66,98±0,31	64,52±6,30	72,95±1,18
500	75,73±2,91	60,17±0,00	-	64,75±2,68	44,84±4,18	68,09±4,10	67,03±8,28

The use of wet SBY created problems in ensuring the homogeneity conditions, as the soluble protein content and antioxidant activity of some control samples was higher than of the samples exposed to high pressure treatment. In tests where the samples were homogenous, the exposure of yeast to high pressure did not lead to cell wall rupture and release of the estimated protein content. The conclusion of this test was that high pressure treatment under the operated conditions is not an effective method of cell wall rupture and the release of intracellular components is not favoured.

Lysis assisted by ultrasound treatment

The ultrasound treatment was performed using a Bandelin, Sonoplus HD 3100 that operates at 200 W and 20 kHz. In this experiment, pulsation mode, amplitude and time were varied (Table 3). After ultrasound treatment, the suspension was centrifuged and the resulting supernatant was analysed for dry matter, soluble protein content, Z-disintegration index and antioxidant activity (ABTS and DPPH method).

Table 3. The conditions applied for performing ultrasound treatment

Amplitude, %	Time min	Pulsation mode
20	2,5	Off
20	5	Off
20	7,5	Off
50	2,5	Off
50	5	Off
50	7,5	Off
70	2,5	Off
70	5	Off
70	7,5	Off
20	2,5	On
20	5	On
20	7,5	On
50	2,5	On
50	5	On
50	7,5	On
70	2,5	On
70	5	On
70	7,5	On

The disintegration index Z showed that higher amplitudes and longer holding times do not exert a positive effect on yeast cell disintegration. At low amplitudes there were no significant differences in the degree of Z score between continuous and pulsed ultrasound treatment. Ultrasonication performed under pulsation mode, and high amplitudes caused lower disintegration of yeast cells.

Dry matter content of the native sample was about 29.9 g/100 g dry yeast. The dry matter content increased during ultrasound treatment, with increasing amplitude and exposure time. The highest dry matter content was obtained when ultrasound treatment was conducted under pulsation mode at 70% amplitude for 7.5 minutes of exposure. (40,02±0,13 g/100 g dry yeast). In order to obtain a high dry matter content, the ultrasound treatment should be performed at high amplitudes and long exposure time under pulsation mode.

The soluble protein content was obtained for samples exposed to ultrasound under pulsation mode, at 70% amplitude, for 5 minutes and the lowest at 50% amplitude.

The antioxidant activity was measured using DPPH and ABTS method. The highest inhibition rates were obtained by using ABTS method and the lowest when using DPPH method. The ultrasound treatment applied for 2.5 minutes gave the highest antioxidant activity at 50% amplitude.

The proteins for yeast suspension exposed to ultrasound treatment have been analysed also by using fluorescence spectroscopy method to assess changes of the Trp and Tyr residues. Shifts in maximum emission have been observed during ultrasound treatment indicating that amino acids are becoming less exposed.

Among the three cell lysis methods, ultrasound treatment resulted in the highest release of protein content.

Activity 1.2. Testing the combination between conventional and nonthermal technologies to obtain highly functional SBY protein hydrolysates/peptides

Three variants have been tested to obtain peptides/ hydrolysates from yeasts

Variant 1

Steps:

1) Preparation of 12 % yeast suspension;
2) Homogenization with Ultraturax at 15.000 rpm, for 10 minutes;
3) Autolysis at 70°C, for 1 hour;
4) Adjustment at pH 7,0;
5) Enzymatic hydrolysis using bromeline, neutrase and trypsin at the following hydrolysis conditions: temperature 50°C, time between 0 - 67 h;
6) Cooling to room temperature and pH adjustment to 7.0;
7) Enzyme inactivation;
8) Centrifugation at 9000 rpm, 10 min;
9) Hudrolysate characterisation.

Variant 2

Steps:

1) Preparation of 12 % yeast suspension;
2) Homogenization with ultrasounds at 70% amplitude, under continuous mode, for 30 minutes;
3) Ajustare suspensie la pH 7,0;
4) Adjustment at pH 7,0;
5) Enzymatic hydrolysis using bromeline, neutrase and trypsin at the following hydrolysis conditions: temperature 50°C, time between 0 - 67 h;
6) Cooling to room temperature and pH adjustment to 7.0;
7) Enzyme inactivation;
8) Centrifugation at 9000 rpm, 10 min;
9) Hudrolysate characterisation.

Variant 3

Steps:

1) Preparation of 12 % yeast suspension;
2) Pretreatment with EnzymeBrew, for 60 minutes, at 55°C;
3) Adjustment at pH 7,0;
4) Enzymatic hydrolysis using bromeline, neutrase and trypsin at the following hydrolysis conditions: temperature 50°C, time between 0 - 67 h;

5) Cooling to room temperature and pH adjustment to 7.0;
6) Enzyme inactivation;
7) Centrifugation at 9000 rpm, 10 min;
8) Hydrolysate characterisation.

The hydrolysates have been characterized for:

- Hydrolysis degree using OPA method
- Colour using L^*, a^*, b^* coefficients
- Dry matter content using reference method
- Soluble protein content using Lowry method
- Antioxidant activity by using DPPH and ABTS method

The type of variant, and the enzyme used for hydrolysis influenced all the parameters considered for characterisation of the yeast samples. The degree of hydrolysis was influenced by the time and type of enzyme, the highest degree of hydrolysis being obtained after 67 hours of hydrolysis using neutrase. The dry matter content, soluble proteins and antioxidant activity increased with increasing time, the highest values were obtained when neutrase was used in enzymatic hydrolysis. The results have shown that there were no significant differences between variant 1 and variant 2, whereas variant 3 under parameters operated did not conduct to the protein hydrolysis (Table 3) and did not cause any changes in the antioxidant activity (Table 4 a, b).

Table 4. The influence of time and enzyme type on the degree of hydrolysis (%) of yeast samples

Sample type	Hydrolysis time (hours)		
	2	4	8
Control	8,22±0,43	8,78±0,07	8,43±0,46
Control with Enzyme brew	8,91±0,65	8,32±0,35	8,17±0,13
Hydrolysate with bromeline	6,9±0,05	9,02±1,04	9,06±0,09
Hydrolysate with neutrase	9,58±0,82	9,5±0,65	9,75±1,03
Hydrolysate with trypsin	9,23±0,03	9,25±1,33	8,69±0,44

Table 4. The influence of time and enzyme type on the antioxidant activity of yeast samples using DPPH (a) and ABTS method (b) (µM Trolox/ g dried yeast)

a)

Sample type	Hydrolysis time (hours)		
	2	4	8
Control	48,82±0,01	34,42±0,01	44,51±0,01
Control with Enzyme brew	55,23±0,01	57,12±0,01	31,9±0,01
Hydrolysate with bromeline	44,20±0,01	36,94±0,01	24,61±0,01
Hydrolysate with neutrase	49,53±0,01	39,46±0,01	35,54±0,01
Hydrolysate with trypsin	53,09±0,01	45,63±0,01	36,10±0,0

b)

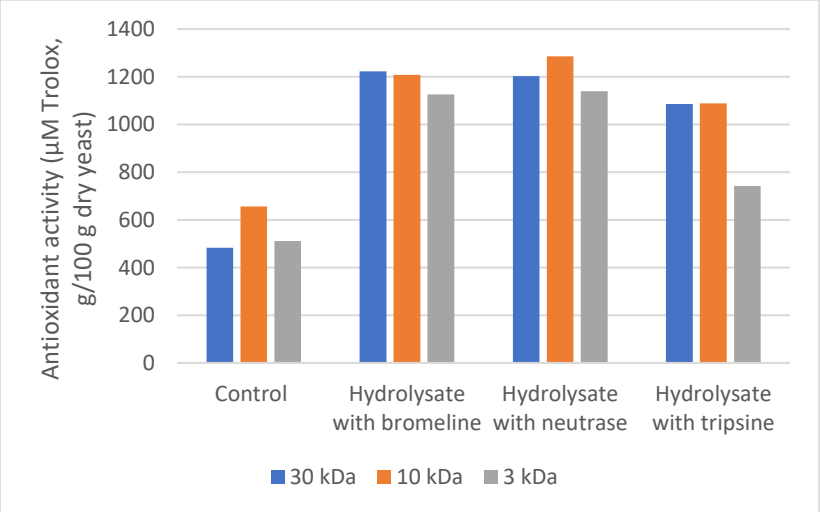
Sample type	Hydrolysis time (hours)		
	2	4	8
Control	661±17	619±2	685±62
Control with Enzyme brew	659±1	711±26	662±18
Hydrolysate with bromeline	645±22	713±9	627±67
Hydrolysate with neutrase	707±57	686±18	724±30
Hydrolysate with trypsin	659±10	690±22	692±25

The following conclusions were drawn from the testing of the 3 variants:

- For the first two variants tested, the degree of hydrolysis, dry matter, soluble protein content and antioxidant activity varied according to the type of enzyme used and the time of hydrolysis;
- There were no significant differences between variant 1 and 2 in terms of the characteristics tested, therefore, it was decided to continue the experiment using variant 1 for obtaining the hydrolysates;
- The enzymatic hydrolysis performed directly on the supernatant did not show significant changes compared to control sample, therefore it was no longer considered for performing the next experiments.

The next step consisted in checking if the antioxidant activity from hydrolysates obtained after 67 h of hydrolysis with neutrase is associated to the formation of peptides. The supernatant was separated using membranes with 30 kDa, 10 kDa and 3 kDa. Gel electrophoresis indicated the presence of peptides, whereas, the antioxidant activity measurements (Figure 1) highlighted that the peptides present in the sample possess antioxidant activity.

a)



b)

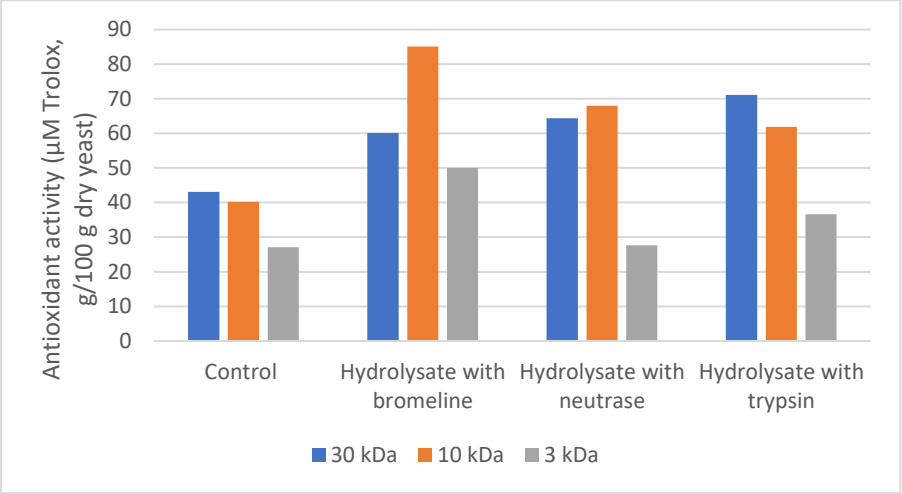


Figure 1. Antioxidant activity of hydrolysates separated with different cut-off membranes using ABTS method (a) and DPPH method (b)

The technological functionality of protein hydrolysates resulted after 67 hours of hydrolysis under the conditions applied for variant 1, have been checked by evaluating the foaming capacity, emulsifying properties and solubility of lyophilised samples.

The assessment of the foaming properties of hydrolysates was performed by using dispersions with a concentration of 6%. The incorporation of air into these dispersions was performed at different homogenization speeds (5000, 7000 and 9000 rpm) for 2 min using the Ultraturax shaker (IKA T18 basic). The foaming capacity was appreciated as the increase in volume of the foam (%) at the end of the homogenization, and the stability of the foam (%) and the collapse of the foam was determined after 15 min and 60 min of keeping at room temperature. Compared to the unhydrolyzed yeast extract that showed an increase in volume of 15% under the conditions of shaking at 5000 rpm, it was noticed that the hydrolysis of yeast proteins with their own enzymes, but especially with exogenous enzymes, led to a significant improvement in foaming properties. For all the analyzed samples it was observed that volume increased with increasing the stirring rate. The protein hydrolysates obtained with trypsin showed the highest values of the increase in volume as well as a very good stability for 15 min of the obtained foams, being followed by the protein hydrolysates obtained with bromeline. The stability of the foams showed values between 62% and 96% even after 60 min of storage at room temperature, but the foams were much less dense compared to the moment of completion of the shaking.

In order to assess the emulsifying properties of yeast protein hydrolysates, emulsions were prepared from mixtures consisting of protein suspension of 6% concentration and sunflower oil (ratio 1:1). The soluble protein content of the hydrolysates subjected to emulsification varied between 2.67% for the hydrolyzed control sample using endogenous enzymes and 6.62% for the protein hydrolysis obtained with neutrase. The protein suspension-oil mixtures were subjected to emulsification in stirring conditions at 5000 rpm for 2 min with the help of the Ultraturax stirrer (IKA T18 basic). The emulsions thus obtained were characterized from a rheological point of view, using the AR 2000ex rheometer, TA Instruments Ltd. Emulsions were subjected to dynamic oscillatory tests using a geometric system of plate-plate type. The emulsions based on protein hydrolysates obtained with neutrase and trypsin presented higher values of G" compared to G' throughout the scanned deformation range, indicating that the more advanced hydrolysis of proteins affects the property of forming firm and stable emulsions.

The rheological tests of forced flow, during which the values of the shear tension (Pa) and viscosity (Pa·s) of the samples were recorded in the conditions of the gradual increase of the shear rate (s-1) in the range of 0.1-100, revealed significant differences between the emulsion-forming properties of the protein hydrolysates of yeast. For all the analyzed samples, the shear tension increased over the entire range of shear speeds tested.

Estimated delivrabiles	Obtained deliverables	Degree of achievement
– 2 hydrolysates with superior functional properties in terms of solubility, emulsifying and antioxidant properties	– 2 hydrolysates with superior functional properties These are obtained through enzymatic hydrolysis of spent brewer yeast of 12% concentration by using neutrase and bromeline for 67 h of hydrolysis.	100%

2. Tuning the techno-functional characteristics of SBY peptides/ proteins/ through controlled glycation assisted by nonthermal technologies

The improvement of the techno-functional characteristics of the proteins/peptides of SBY will be carried out on the hydrolysed samples with bromeline or neutrase obtained using Variant 1. Conjugation will be carried out by using:

a) Conventional wet conjugation, performed under different conditions of reaction time and temperature;
b) Ultrasound assisted wet conjugation, performed by selecting different values of ultrasonication amplitude and time;

There are 4 experimental variants underway, which use maltodextrin as a partner in the Maillard reaction:

Variant 1 – the peptide hydrolysate with the concentration of 4% will be mixed with maltodextrin in a ratio of 1:5 (m/m), in order to carry out the conjugation reaction at pH 7.0, for different time intervals, at a temperature of 75 °C.

Variant 2 - the peptide hydrolysate with the concentration of 4% will be mixed with maltodextrin in a ratio of 1:5 (m/m), in order to carry out the conjugation reaction at pH 7.0, by applying ultrasound, at different time intervals.

Variant 3 – yeast cells with a concentration of 4% will be mixed with maltodextrin in a ratio of 1:5 (m/m), in order to carry out the conjugation reaction at pH 7.0, at different time intervals, at 75 °C.

Variant 4 - yeast cells with a concentration of 4% will be mixed with maltodextrin in a ratio of 1:5 (m/m), in order to carry out the conjugation reaction at pH 7.0, by applying ultrasounds, at different time intervals.

Estimated result indicators	Obtained result indicators	Grad de îndeplinire
- 1 article submitted to ISI/BDI journal; - Dedicated web page	- 1 article accepted for publication in Paper published in journal indexed in Web of Science - Core Collection. Dumitrascu L., Lanciu (Dorofte) A., Aprodu I. A preliminary study on using ultrasounds for the valorization of spent brewer's yeast. The Annals of the University Dunarea de Jos of Galati – Food Technology, 46(2), 1-15. https://www.gup.ugal.ro/ugaljournals/index.php/food	100%

- Project website www.funyeast.ugal.ro
- Participation to the conference CEFood Slovenia with papers
  1) Aprodu I., Enachi E., Borda D., Dumitrascu L. *Saccharomyces cerevisiae* as source of carrier material for bioactive compounds encapsulation. 11th Central European Congress on Food and Nutrition, 27-30 September 2022. Čatež ob Savi, Slovenia (poster presentation). https://cefood2022.si/
  2) Dumitrascu L. Non-animal protein sources and resources- new strategies of valorization into value-added ingredients. 11th Central European Congress on Food and Nutrition, 27-30 September 2022. Čatež ob Savi, Slovenia (oral presentation). https://cefood2022.si/
- Position paper

Dumitrascu L. Non-animal protein sources and resources- new strategies of valorization into value-added ingredients. Food, nutrition and environment: Position papers in Central European space, pp. 129-138. Publisher: Croatian Academy of Engineering, Zagreb, ISBN: 978-953-7076-30-6.