# Lysates of *Metschnikowia* Yeast with Higher Content of Hydroxyproline

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The chemical characteristics of lysates obtained from yeasts belonging to *Metschnikowia* spp. were determined. Cell lysis was induced using saponin from *Q. saponaria* or 5% NaCl. The process was conducted at 50 °C for 48 h. The enzymatic profiles of the resulting lysates were analyzed. The mannose and glucose contents were also investigated, as well as the concentrations of proteins, free amino nitrogen (FAN), and free amino acids. The results were compared to the characteristics of lysates from conventional industrial strains of *Saccharomyces* spp. obtained under analogous conditions. The *Metschnikowia* lysates showed different chemical profiles and the pool of individual amino acids was generally smaller. However, the content of hydroxyproline HPro was 4 to 5 times higher. The results of this study show that yeast lysates are an attractive supplement for numerous applications.

*Keywords: Metschnikowia; Autolysates; Amino-acid profile; Hydroxyproline*

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## INTRODUCTION

Yeast lysates are used in the medical, food, and cosmetics industries. They are a source of carbohydrates, proteins, and free amino acids, which may be used as substrates for the biosynthesis of various compounds, such as flavors, hormones, coenzymes, or neurotransmitters. Yeast lysates are obtained mainly from yeast of the genus *Saccharomyces*. Yeast propagated for this purpose, or post-fermentation yeast, left over from the production of beer or wine, may be used. It is then submitted to slow natural autolysis or a much faster induced process. Induced lysis is deliberately caused by the effects of temperature, high pressure homogenization, ultrasound treatment, or chemical inducers (enzymes, detergents, salts, or alcohols). Rarely, other industrial yeasts from the genera *Kluyveromyces*, (Lukondeh *et al*. 2003; Berłowska *et al.* 2015), *Scheffersomyces*/ *Pichia* (Bartolo-Aguilar *et al.* 2017; Berłowska *et al.* 2017), or *Yarrowia* (Pozo-Dengra *et al.* 2006) may be utilized. The chemical composition of the obtained lysates depends on the method of cell disruption as well as on the genera or species of the yeast (Berłowska *et al.* 2015; Berłowska *et al.* 2017; Comuzzo *et al.* 2017; Bertolo *et al.* 2019; Jacob *et al.* 2019).

The yeast genus *Metschnikowia* is included on the list of biotechnologically useful microorganisms prepared by scientists and the food industry (Bourdichon *et al*. 2012). This non-conventional yeast is recommended for the release of volatile thiols and terpenes in white wines, increasing their aromatic intensity (Mas *et al*. 2016). For example, an increase in the levels of the thiol 4-MSP (4-methyl-4-sulfanylpentan-2-one) and a decrease in higher alcohol content, may give important impact on white wines, making them fruitier and fresher (Ruiz *et al.* 2018). Strains of *Metschnikowia* have been used in the production of

low alcohol wines (Varela *et al.* 2017), and their potential for the deoxidation of fermentation wort has also been recognized (Pawlikowska *et al.* 2019b). *Metschnikowia* strains have been identified as a possible biocontrol agent against *Botrytis* and numerous post-harvest fruit diseases (Sipiczki 2006; Pawlikowska *et al.* 2019a). However, despite the growing interest in *Metschnikowia* strains, there are no data on the characteristics of their autolysates. Therefore, this research studied the enzymatic profiles, proteins, and amino acid profiles of autolysates produced from *Metschnikowia* strains. Autolysis was chemically assisted in both conventional and non-conventional ways, using NaCl and saponins from *Quillaja saponaria* (Berłowska *et al.* 2015; Berłowska *et al.* 2017). The researchers compared the results for *Metschnikowia* spp. with the characteristics of yeast autolysates from classical strains of *Saccharomyces* spp. This is the first report on the chemical characteristics of *Metschnikowia* spp. lysates.

## EXPERIMENTAL

### Yeast Strains and Cultivation

Biomass from three yeast strains belonging to *Metschnikowia* spp were used: the collection strain *M. pulcherima* NCYC2321 (Norwich, UK) and two isolates, *M. andauensis* D2 (GenBank MK612095), and *M. sinensis* D9 (GenBank MK612102) (Pawlikowska *et al.* 2019a). Two strains of *Saccharomyces* spp. were also utilized: the winery strain *S. cerevisiae* Tokay, and the brewery yeast *S. cerevisiae* TT from LOCK105 Culture Collection (Lodz, Poland). The yeasts were cultured in 50 mL of wort broth (Merck) in 500 mL round bottom flasks at 25 °C for 72 h on a rotary shaker at 220 rpm. After cultivation, the cells were washed twice with sterile distilled water by centrifugation (10 °C, 10 min, 3000 × g, Eppendorf 5804R, Hamburg, Germany). The concentration of yeast cells was determined via counting with a hemocytometer and standardized to $10^9$ cells/mL.

### Induced Lysis

The yeast biomass was suspended in distilled water with a ratio of 1:1. Saponins from *Q. saponaria* (Sigma Aldrich, St. Louis, MO, USA) were then added to a final concentration of 0.08% w/v, as an inducer of cell lysis. The cell lysis was conducted in 500 mL Erlenmeyer flasks on a laboratory shaker (112 rpm) at 52 ± 2 °C for 48 h (Heidolph Unimax 1010, Schwabach, Germany). Then, the solid residue was centrifuged (5 °C, 15 min, 3500×g; Eppendorf 5804R, Hamburg, Germany) and the supernatant was collected.

### Protein Concentration

The protein content in the yeast lysates was analyzed using a Direct Detect® System (Merck–Millipore, Waltham, MA, USA). This method is based on Fourier transform infrared spectroscopy (FTIR), which detects amide bonds in polypeptide chains (Berlowska *et al*. 2017).

### FAN Determination

The free amino nitrogen concentration (FAN) was determined based on the color reaction of amino acids with ninhydrin and by absorbance measurements at a wavelength of 570 nm. The concentration of ammonia nitrogen was measured using the colorimetric method with Nessler's reagent at a wavelength of 400/425 nm (Berlowska *et al.* 2015).

### Free Amino Acid Profiles

To remove the protein fraction with a molecular weight over 3 kDa, the yeast lysate was centrifuged at 25 °C, speed 10,000 g, 2 h; 5804R (Eppendorf, Hamburg, Germany) and filtered using Amicon® Ultra-4 Ultracel-3 membranes (Merck–Millipore, Massachusetts, USA). The samples were then derivatized using phenylisothiocyanate to form phenylthiocarbamyl amino acids (Waters Workstation, Massachusetts, USA). The derivatived forms were analyzed by HPLC (diluent WAT088119, Eluent A WAT052890, Eluent B WAT088112, Pico Column 3.9 × 300 mm, time 20 min; Waters, Waltham, MA, USA, Thermo Finnigan Surveyor HPLC System, Waltham, MA, USA). The amino acids content were expressed in pmol units (Berlowska *et al.* 2017).

### Enzymatic Profiles

The enzymatic profiles of the yeast suspensions, including 19 different enzymes, were estimated after 24 h of autolysis using the API ZYM test (BioMerieux, Lyon, France). Inoculation and evaluation were carried out based on the manufacturer's instructions and recommendations. Only those suspensions that demonstrated visible changes in the color of the medium were considered to show enzymatic activity. The intensity of the color reflected the amount of degraded substrate. Values ranged from '0', no activity, to '3', maximum activity (Pawlikowska *et al.* 2019a).

### Mannose and Glucose Content

The monosaccharide profiles of the obtained yeast lysates were analyzed using a UV-spectrophotometer (Thermo Scientific Multiskan GO; Thermo Fisher Scientific, Munich, Germany) and a Megazyme K-MANGL Kit for glucose and mannose determination. The assays were conducted according to the manufacturer's instructions (Berlowska *et al.* 2017).

### Statistics

Means were calculated from the data obtained from three independent experiments and the standard deviation (SD) was calculated.

## RESULTS AND DISCUSSION

In this study, researchers used both classical yeasts belonging to *Saccharomyces* spp. and non-conventional yeasts from *Metschnikowia* spp. Solutions of NaCl and plant saponin from *Q. saponaria* were used as lysis inducers. In previous studies on yeast lysis conducted by Berlowska *et al*. (2017), it was observed that saponins from *Q. saponaria* promote the autolysis of different yeast genera: *Saccharomyces, Kluyveromyces, Scheffersomyces*, and *Pichia*. This analysis studied the activities of the enzymes in yeast suspensions undergoing induced lysis. Table 1 presents the results of enzymatic fingerprinting obtained after 24 h of incubation, in the middle of the lysis process.

The use of API Zym enabled assessment of the activity of the enzymes responsible for catalyzing protein hydrolysis (trypsin, α-chymotrypsin, leucine arylamidase, valine arylamidase, and cystine arylamidase), ester bond hydrolysis (esterase C4, esterase, and lipase), dephosphorylation reactions (alkaline phosphatase, acid phosphatase, and naphthyl-AS-BI-phosphohydrolase).

**Table 1.** Enzymatic Activity of Yeast Cell Suspensions Determined by the Api Zym Assay

| Enzyme | | Yeast strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Classes | Specific activity | NCYC | | D2 | | D9 | | Tokay | | TT | |
| | | NaCl | S | NaCl | S | NaCl | S | NaCl | S | NaCl | S |
| Proteases | Leucine arylamidase | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Valine arylamidase | 0 | 0 | 1 | 1 | 0 | 0 | 3 | 3 | 3 | 3 |
| | Cystine arylamidase | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| | Trypsin | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lipases | Esterase (C4) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 |
| | Esterase lipase | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Phosphatases | Alkaline phosphatase | 2 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 |
| | Acid phosphatase | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 1 | 1 |
| | Naphtol-AS-BI-phosphohydrolase | 2 | 1 | 1 | 2 | 1 | 1 | 3 | 3 | 3 | 3 |
| Glycosidases | α-Galactosidase | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 1 |
| | β-Glucuronidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | α-Glucosidase | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 |
| | α-Mannosidase | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| S, saponin; 0, negative; 1, weak positive; 2, moderate positive; 3, strong positive | | | | | | | | | | | |

The assay also evaluated binding of carbohydrate (α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase). During autolysis, researchers noted the highest activity of phosphatases in the *Metschnikowia* spp. suspensions. Recent insights into the function and regulation of these enzymes have unveiled extremely interesting aspects of yeast biology. For example, phosphatases regulate salt tolerance, G1/S cell cycle transition, and cell integrity, as well as being involved in protein synthesis (Arino 2002). Their activity, which was significant in the middle of the autolysis process, indicates the occurrence of many cell defense processes under strong environmental stress (temperature, osmotic pressure, and cell density). In the autolyzed mixtures, the activity of arylamidases was observed, which proves the occurrence of protein hydrolysis. In the yeast mixtures, no glycosidase activity was detected.

The enzymatic profiles of the conventional yeast strains varied. In *Saccharomyces* spp. suspensions researchers noted higher activity for some proteases (valine arylamidase and cystine arylamidase), lipases (esterase C4 and esterase), and some carbohydrate-binding enzymes (α-galactosidase, β-glucuronidase, α-glucosidase, and α-mannosidase). Very high activity was observed for one phosphatase using naphthol-AS-BI-phosphate. These activities indicate the progressive degradation of proteins, lipids, and carbohydrates, namely glucanes and mannanes – the main constituents of yeast cell wall (Berlowska *et al*. 2016).

After the final process of autolysis, the contents of proteins, free amino nitrogen (FAN), and monosaccharides (glucose and mannose) in the yeast lysates were measured. The results are presented in Table 2.

**Table 2.** General Characteristics of Yeast Lysates

| Yeast strain | Lysis inducer | Protein (mg/mL) | FAN (mg/mL) |
|---|---|---|---|
| *M. pulcherrima* NCYC2321 | NaCl | 4.48 ± 0.56 | 1131.34 ± 23.45 |
| | saponin | 3.51 ± 0.26 | 831.34 ± 67.34 |
| *M. andauensis* D2 | NaCl | 5.48 ± 0.24 | 1127.34 ± 45.67 |
| | saponin | 2.53 ± 0.09 | 1029.34 ± 67.65 |
| *M. sinensis* D9 | NaCl | 4.76 ± 0.35 | 656.66 ± 45.34 |
| | saponin | 3.16 ± 0.12 | 666.66 ± 56.78 |
| *S. cerevisiae* Tokay | NaCl | 8.59 ± 0.34 | 463 ± 46.08 |
| | saponin | 6.91 ± 0.36 | 883 ± 66.73 |
| *S. cerevisiae* TT | NaCl | 7.87 ± 0.22 | 2102.33 ± 76.78 |
| | saponin | 3.38 ± 0.16 | 1654 ± 66.13 |

The characteristics of individual yeast strains were comparable, regardless of the inductor used. The activity of glucanases in the case of *Saccharomyces* spp. resulted in increased content of mannose and glucose equal to 11 mg/mL and within the range 33 to 85 mg/mL after action of saponin, respectively. In the case of NaCl treatment, carbohydrate content was lower (9.5 and 29 to 56 mg/mL, respectively). It is worth noting that the mannose and glucose content in *Metschnikowia* spp. was close to zero if no glucanase activity detected. The protein and FAN contents were also both strain- and inducer-dependent. More protein was determined in the lysates obtained with NaCl. These results differ from those of earlier studies for the strain *Saccharomyces cerevisiae* with Ethanol Red® (Lesaffre) by Berlowska *et al*. (2015).

Table 3 shows the amino-acid profiles of two yeast strains, *M. andauensis* D2 and *S. cerevisiae* TT, obtained by NaCl or saponin treatment. These yeasts were chosen due to the fact that their lysates contained the highest amounts of proteins and FAN (Table 2).

**Table 3.** Amino Acid Profiles of Yeast Lysates

| Amino acid (pmol) | D2 | | TT | |
|---|---|---|---|---|
| | NaCl | Saponin | NaCl | saponin |
| Asp | 17.15 ± 0.16 | 141.11 ± 9.46 | 562.98 ± 7.12 | 587.12 ± 37.96 |
| Glu | 591.60 ± 14.11 | 1624.44 ± 17.02 | 1034.59 ± 45.23 | 1077.04 ± 49.19 |
| **HPro** | **3.03 ± 0.06** | **4.26 ± 0.11** | **0.72 ± 0.07** | **0.80 ± 0.05** |
| Ser | 218.14 ± 7.10 | 49.01 ± 6.12 | 778.93 ± 65.11 | 797.93 ± 43.09 |
| Gly | 450.34 ± 12.16 | 410.37 ± 4.11 | 1019.29 ± 89.56 | 1057.36 ± 76.75 |
| His | 495.31 ± 14.19 | 391.87 ± 7.36 | 477.46 ± 32.06 | 497.64 ± 24.16 |
| Arg | 770.45 ± 30.17 | 4.45 ± 0.16 | 760.04 ± 27.78 | 731.86 ± 40.11 |
| Thr | 238.92 ± 22.10 | 84.58 ± 7.13 | 1160.43 ± 58.13 | 1060.62 ± 65.06 |
| Ala | 1496.34 ± 87.12 | 1075.78 ± 16.17 | 2301.05 ± 79.37 | 2298.63 ± 97.19 |
| Pro | **1135.27 ± 38.13** | **1333.28 ± 64.16** | **967.04 ± 87.76** | **1011.17 ± 55.16** |
| Tyr | 202.31 ± 9.16 | 88.71 ± 3,11 | 341.47 ± 19.10 | 340.77 ± 10.16 |
| Val | 1067.71 ± 26.14 | 752,30 ± 12.06 | 2336,99 ± 97,72 | 2432.03 ± 123.09 |
| Met | 79.81 ± 8.16 | 65.97 ± 7.10 | 535.86 ± 13.14 | 557.21 ± 54.14 |
| Cys-Cys | 22.35 ± 7.12 | 36.40 ± 2.16 | 27.22 ± 4.19 | 28.03 ± 2.46 |
| Ile | 676.32 ± 32.16 | 413.97 ± 10.18 | 1975.91 ± 43.17 | 2054.94 ± 100.12 |
| Leu | 1067.71 ± 53.17 | 708.52 ± 14.13 | 2719.50 ± 99.03 | 2831.51 ± 45.16 |
| Phe | 265.49 ± 11.11 | 116.84 ± 9.06 | 1294.31 ± 42.07 | 1310.80 ± 63.34 |
| Lys | 736.02 ± 15.12 | 183.87 ± 7.89 | 477.27 ± 26.16 | 497.51 ± 31.17 |
| **Sum** | **9534,27** | **7485,73** | **18771,06** | **19172,97** |

The autolysates of the yeast *Metschnikowia* sp. show a different chemical profile for free amino acids. The pool of amino acids in the D2 autolysate was two times lower than that of the TT autolysate. In general, the obtained amino acid profiles of *Metschnikowia* strain were dominated by alanine, proline, valine, and leucine, while in *Saccharomyces* autolizate, mainly glutamic acid, glycine, threonine, alanine, valine, isoleucine, leucine and phenylalanine were detected. The both yeast lysates obtained were found to be good sources of free essential amino acids. These valuable compounds accounted for 43% (for *M. andauensis*), and 56% (for *S. cerevisiae*) of the respective amino acid pools. However, it is interesting that the content of hydroxyproline HPro in the *Metschnikowia* sp. preparation was 4 to 5 times higher. Hydroxyproline-rich glycoproteins are key protein constituents of the cell wall (Lee *et al.* 2007). Recent discoveries have revealed that hydroxyproline and proline play important roles in growth and differentiation across the life cycle. These amino acids are important components of cell wall proteins, which play pivotal roles in cell wall signal transduction cascades, cell development, and stress tolerance (Nasuno *et al.* 2013; Kavi Kishor *et al.* 2015). Proline and hydroxyproline not only participate in protein synthesis, but also regulate several important functions including replicative lifespan, osmotic adjustment, and protection during stress conditions (Mukai *et al*. 2019). *Metschnikowia* spp. are known for their unique adaptive abilities, and in this respect differ significantly from the more sensitive *Saccharomyces* spp. (Pawlikowska and Kregiel 2017). Deficient proline biosynthesis leads to abnormal cells and cell wall defects, indicating its important role in the formation of structural proteins (Kishor *et al.* 2015). In *Metschnikowia* sp., proline increases the production of a pigment,

pulcherrimin, which improves tolerance to oxidative stress and biocontrol (Liu *et al.* 2019; Pawlikowska *et al.* 2019b). It is worth noting that *Metschnikowia* strains show high antifungal activity, which means they compete with other fungal microflora (Sipiczki 2006; Pawlikowska *et al.* 2019a). These data may explain the increased hydroxyproline content of *Metschnikowia* yeast lysates.

Lysates of *Metschnikowia* spp. with a high hydroxyproline content may find applications in biotechnology. Proline regulates several important functions in higher plants, such as osmotic adjustment and protection under stress conditions (Kishor *et al.* 2015). Hydroxyproline and proline act as biostimulants, penetrating rapidly into plant tissues. The mode of action of complex preparations is varied and may include the activation of nitrogen metabolism, phosphorus release from soils, generic stimulation of soil microbial activity or the stimulation of root growth, and enhanced plant establishment (Yakhin *et al.* 2017). Proline and hydroxyproline can be considered as functional amino acids for mammalian, avian, and aquatic species. Research on young pigs has shown that proline supplementation improved the daily growth rate and feed efficiency. Dietary supplementation of a plant protein-based diet with hydroxyproline additionally enhanced weight gain in salmon (Wu *et al.* 2011). Hydroxyproline is the main component of collagen in mammals (Gordon and Hahn 2010), and both proline and hydroxyproline are critical to the mechanical strength of this extracellular matrix molecule. Therefore, *Metschnikowia* spp. lysates could also be used in the cosmetic industry as regenerating, revitalizing, smoothing, and moisturizing agents (Asserin *et al.* 2015).

## CONCLUSIONS

1. *Metschnikowia* lysates show different chemical profiles in comparison to *Saccharomyces* spp. The pool of individual amino acids was generally lower. However, the content of hydroxyproline was 4 to 5 times higher.
2. *Metschnikowia* sp. lysates can be used in the form of mono-autolysates or can enrich autolysates of the classical yeast *S. cerevisiae* deficient in hydroxyproline.
3. Lysates of *Metschnikowia* sp. with high hydroxyproline contents may find application in biotechnology, particularly in the agriculture and cosmetics industries.

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