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# DEGRADATION OF FLAVONOIDS BY *Cryptococcus albidus* α-L-RHAMNOSIDASE

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The aim of the work was to investigate the practical using of α-L-rhamnosidase substrate specificity *Cryptococcus albidus*. *p*-Nitrophenyl derivatives of monosaccharides were used to determine the activity and the enzyme specificity. The ability to hydrolyze of natural substrates was evaluated by Davis and high-performance liquid chromatography methods. It was shown that the enzyme exhibited narrow specificity towards the glycon of synthetic substrates and hydrolyzes only *p*-nitrophenyl-α-L-rhamnopyranoside ($K_m$ 4.5 mM) and *p*-nitrophenyl-α-D-glucopyranoside ($K_m$ 10.0 mM). *C. albidus* α-L-rhamnosidase the most active degrades naringin ($K_m$ 0.77 mM), releasing prunin and naringenin. $K_m$ for neohesperidin was 3.3 mM. The efficacy of the naringin hydrolysis in grapefruit and pomelo juice was 98 and 94% in 60 min (40 °C, 2 U/ml). As the result of of green tea and orange juice treatment by α-L-rhamnosidase, there was a decrease in the content of rutin, narirutin and hesperidin, indicating that α-1,2- and α-1,6-linked rhamnose could be cleaved from natural flavonoids. The study shows the possibility of citrus juices and green tea treatment by *C. albidus* α-L-rhamnosidase for the purpose of their taste qualities improvment and obtaining bioavailable flavonoids glucosides.

***Key words:*** *Cryptococcus albidus*, α-L-rhamnosidase, naringin, neohesperidin, rutin, flavonoids, citrus juices, green tea.

Currently, O-glycosyl hydrolases (enzymes able to degrade the glycoside bond) are employed in more and more biotechnological processes, more only to proteases. Most such enzymes are used to treat various plant-derived materials. Among them, an important place belongs to α-L-rhamnosidase (α-L-rhamnoside-rhamnohydrolase — E.C. 3.2.1.40), which splitted off the terminal unreduced α-1,2-, α-1,4- and α-1,6- bound L-rhamnose, present in natural glycoconjugates and synthetic glycosides. Splitting the O-glycoside bond occurs with preservation of the configuration of the anomeric carbon atom ($C_1$ in the cyclic form of the monosaccharide). Natural substrates for the α-L-rhamnosidases are plant glyscosides, glycolipids, gums, pigments, resins, specific immunopolysaccharides, heteropolysaccharides of bacterial cell walls, saponins, glycoalkaloids, pectins, flavonoids of many plants: grapes, citrus crops, green tea, buckwheat, *Sophora japonica*, *Rosa* sp., *Sorbus* sp., *Prunus* sp., pepper, etc. [1, 2]. Plant bioflavonoids, the most common type of natural polyphenolic compounds, have a special place among possible substrates for α-L-rhamnosidases. Flavonoids incite quite understandable interest, since many studies show their valuable properties for supporting and improving human health; recently, they were used to create certain cardio-vascular drugs and antiviral and immunotropic agents [3]. Yet the bio-accessibility of such flavonoids for humans is rather limited, due to the presence of carbohydrates (rhamnose, glucose and galactose) [4]. The rutinoside component (rhamnose+glucose) of flavonoids in many plant products, was found to hinder their intake in the gut [5]. The strong interest to α-L-rhamnosidase is caused exactly by its ability to split off the terminal rhamnose, thus opening a wide field of possible applications of the enzyme for biodegradation of flavonoids.

The rhamnose binds to glucose at different positions (α-1,2-, α-1,4-, α-1,6 -) in different flavonoid glycosides, and glucose itself has to bind to the aglycone at the third or the seventh carbon. Thus, selective glycosidases

are needed to cleave the glycoside bonds both inside the disaccharide and between the carbohydrate fragment and the flavonoid aglycone. Hence, studying substrate specificity of α-L-rhamnosidase on various substrates is an important stage of developing efficient preparations for the biotransformation of plant polyphenols and their further use. For example, in the pharmaceutical industry, α-L-rhamnosidase is applied to rutin and hesperidin to obtain many medical substances based on the flavonoid glycosides and deglycosylated flavonoids [6]. Using α-L-rhamnosidases in food industry is aimed at improving the quality of drinks (decreasing the bitterness of citrus juices, enhancing the wine aromas) and making food additives (various biopolymers and sweeteners) [7]. As a byproduct of enzymatic hydrolysis of plant glycosides, the rhamnose can be accumulated in large quantities and used afterwards to produce rhamnolipids for cosmetics.

There are reports of a few microbial vigorously α-L-rhamnosidases which have specificity to certain flavonoids. These enzymes of *Aspergillus* and *Penicillium* were highly specific to rutin and naringin [8–11]. The ability to hydrolyze naringin, hesperidin, rutin, narcissin was found for probiotic bacteriae vigorously [12, 13] and bacilli [14]. A few yeast sources were also described of α-L-rhamnosidase with high biotechnologic potential [15].

We isolated the enzyme from yeast *Cryptococcus albidus* and studied some of its physico-chemical, kinetic and catalytic properties [16]. Our current study was aimed at substrate specificity of *C. albidus* α-L-rhamnosidase towards some synthetic and natural substances, and at estimating the enzyme's ability to degrade flavonoids in citrus juices and green tea.

## Materials and Methods

α-L-rhamnosidase preparation was obtained from the supernatant of the *C. albidus* cultural liquid by precipitation in ammonium sulfate (to 90% saturation) followed by chromatography on charged and neutral TSK-gels (DEAE-Toyopearl 650-s and Toyopearl HW-60 "Toya Soda" Japan, respectively) [16]. Specific α-L-rhamnosidase activity of the preparation was 12 units/ mg protein.

The glycosidases activities were determined using the synthetic substrates: n-nitrophenyl-α-L-rhamnopyranoside, *n*-nitrophenyl-α- and β-D-galactopyranoside; *n*-nitrophenyl-α- and β-D-glucopyranoside; *n*-nitrophenyl-N-acetyl-β-D-galactopyranoside; *n*-nitrophenyl-N-acetyl-α- and β-D-glucopyranoside; *n*-nitrophenyl-β-D-glucuronide; *n*-nitrophenyl-β-D-xylopyranoside; *n*-nitrophenyl-α-D-mannopyranoside; *n*-nitrophenyl-α-D-fucopyranoside (Sigma-Aldrich, USA).

To assay the glycosidase activity, 0.1 ml of enzyme solution was mixed with 0.2 ml 0.1 M phosphate-citrate buffer (PCB) of pH 5.2 and 0.1 ml 0.01 M solution of the substrate in PCB. The mixture was incubated for 10 min at 37 °C. The reaction was stopped by adding 2 ml 1 M solution of sodium bicarbonate. To control samples, the same components were added, but in reverse. The amount of released *n*-nitrophenol as a result of hydrolysis was determined colorimetrically at 400 nm [17]. One unit of enzyme activity was defined as the amount of the enzyme which hydrolyzed 1 μmol substrate per minute.

The α-L-rhamnosidase activity was followed Davis method[18] using natural substrates (naringin, neohesperidin). Reaction mixture comprised 1 ml 0.05 % naringin or neohesperidin in 0.1 M PCB with pH 5.2 and 1 ml enzyme solution. The mixture was incubated for 60 min at 37 °C. To each aliquot of 0.2 ml, we added 10 ml diethylenglycole and 0.2 ml 4M NaOH. The mixture was kept at room temperature for 10 min, and the intensity of yellow coloring was measured spectrophotometrically at 420 nm. One unit of activity was defined as the amount of enzyme which releases 1 μmol substrate per minute.

Maximal velocity ($V_{max}$) and Michaelis constant ($K_m$) were determined by the Lineweaver — Burk plots [19].

Grapefruit, orange and pomelo juices were obtained from fresh fruit. Juice samples were extracted and filtered to exclude seeds and skins. Standard flavonoid solutions (rutin, neohesperidin, naringenin, quercetin, prunin, narirutin, hesperidin) were prepared in concentration of 1 mg in 1 ml 50% ethanol. Enzyme preparation was dissolved in 0.1 M PCB at 1 mg/ml (12 units). 0.1 ml enzyme solution was added to 2 ml juice and incubated at 40 °C for 60 min. Control samples had 0.1 ml 0.1 M PCB+2 ml juice. The reaction was stopped by adding 1 ml anhydrous ethanol to 2 ml of the sample and vigorously mixing. Then, the samples were centrifuged at 10000 rpm for 10 min, the supernatant was filtered and analyzed by high performance liquid chromatography (HPLC).

Green tea leaves were treated in the following way. 1.5 g of tea was boiled for 5 min in 150 ml water with 0.5 ml dimethyl sulfoxide.

Then the samples were processed as above. The control sample had 0.1 ml 0.1 M PCB to 2 ml tea solution.

Quantitative and qualitative flavonoid analysis was done on liquid chromatographer Agilent 1200 HPLC with a diode matrix detector at 280 nm. Samples were injected into column Zorbax SB C18 (2.5×150 mm; 3.5 μm). Thermostat temperature was 27 °C. The calibration curves were built by used the commercial flavonoid preparations (Sigma-Aldrich, USA).

The quantitative and qualitative analysis of flavonoids was carried out in the Center for collective equipment use at the Institute for microbiology and virology of NAS of Ukraine.

All experiments were replicated 5–8 times. Analysis of the data was done using Student's *t*-criterion. The results, presented graphically, were obtained using the Microsoft Exel 2003 software. Values were considered significant at $P < 0.05$.

## Results and Discussion

Currently, the enzymatic degradation of carbohydrate-linked flavonoids is considered one of efficient methods to obtain the biologically active substances for pharmaceutics, medicine and food production [2, 3, 6, 7]. Microbial naringinases and α-L-rhamnosidases are able to bioconvert flavonoids safely [8, 9]. There are reports of highly active sources of such enzymes, and their biotechnological potential is studied with regards to substrate specificity [20].

According to literature, various α-L-rhamnosidases are able to hydrolyze the α-1,2, α-1,3-, α-1,4- and α-1,6-glycoside bonds and show higher affinity to plant flavonoids compared to the synthetic analogues [2, 20]. $К_m$ for *n*-nitrophenyl-α-L-rhamnoside is from 0.057 to 2.8 mM, for naringin it is 0.021–1.9 mM, for hesperidin 0.02–1.3 mM, for rutin 0.028–1.44 mM, for quercitrin 0.077–0.89 mM, and for poncirin 0.02–0.93 мМ.

We studied substrate specificity of *C. albidus* α-L-rhamnosidase both for the synthetic *n*-nitrophenyl derivatives of monosaccharides and for the natural flavonoids such as naringin and neohesperidin. α-L-Rhamnosidase of *C. albidus* had higher affinity to naringin and neohesperidin than to synthetic analogues, according to the respective values for $К_m$ and $V_{max}/K_m$ for the hydrolysis of the substances (Table). Such specificity is characteristic for other yeast α-L-rhamnosidases [15, 20]. As to the synthetic derivatives of monosaccharides, it was narrow specific towards glycon; we showed that the enzyme was able to cleave only *n*-nitrophenyl-α-L-rhamnopyranoside and *n*-nitrophenyl-β-D-glucopyranoside (Table).

To evaluate the ability of *C. albidus* α-L-rhamnosidase to biotransform flavonoids we used freshly prepared citrus juices (orange, mandarin, grapefruit and pomelo) as well as green tea. All these drinks have polyphenolic substances which can negatively impact the product taste, or have only limited bioavailability. Thus, large quantities of naringin are the reason why many citrus juices are bitter. They can be removed using naringinases, rutinosidases and α-L-rhamnosidases, which transform naringinin to less bitter prunin and naringenin [1, 7]. The presence of hesperidin and neohesperidin in orange and mandarin juices is the reason why they become cloudy and crystallize. Using the rhamnosidases in their production allows to avoid this and improve the taste. Splitting off the glycon of rutin allows to obtain biologically active isoquercitrin and quercetin, as well as to increase their amount in the product [4, 6, 8–10].

We tested the efficiency of commercial preparations of naringin, neohesperidin, citrus juices and green tea with the *C. albidus* α-L-rhamnosidase. We showed that in all of these cases, the enzyme was able to hydrolyze the flavonoids. α-L-rhamnosidase hydrolysed naringin to prunin and naringenin both in the commercial preparation and in the pomelo and grapefruit juices (Fig. 1, 2). We observed the naringin concentration drop by 98 and 94% of the initial quantity. Prunin production was significantly higher than naringenin production. The latter concentration at the end was 5.4 and 2.5% for grapefruit and pomelo, respectively. Cleaving off of rhamnose and glucose happened gradually as a result of the α-L-rhamnosidase and β-glucosidase activity of the preparation. Also, one-stage cleaving off of the disaccharide rutinoside did not occur. Based on the results of the purification of *C. albidus* α-L-rhamnosidase [16] and the $К_m$ та $V_{max}/K_m$ for the hydrolysis of *n*-nitrophenyl-α-L-rhamnopyranoside and *n*-nitrophenyl-β-D-glucopyranoside, we suppose that this is one of the same enzyme — an α-L-rhamnosidase with nonspecific β-glucosidase activity. Another term for such enzymes is naringinase [7, 14, 20].

Glycoside removal in commercial preparations of naringin and neohesperidin also occurred fairly fast. Thus, after 60 min the flavonoid amount dropped from 500 μg/ml to 20 and 100 μg/ml, respectively. We also noticed that the α-L-rhamnosidase of

**Substrate specificity of *C. albidus* α-L-rhamnosidase**

| Substrate | Bond type | $K_m$, мМ | $V_{max}$, µmol/min/mg | $V_{max}/K_m$ |
|---|---|---|---|---|
| *n*-nitrophenyl-α-L-rhamnopyranoside | α-1 | 4.5 | 15 | 3.3 |
| *n*-nitrophenyl-β-D-glucopyranoside | β-1 | 10 | 5 | 0.5 |
| *n*-nitrophenyl-α-D-galactopyranoside | α-1 | – | 0 | – |
| *n*-nitrophenyl-β-D-galactopyranoside | β-1 | – | 0 | – |
| *n*-nitrophenyl-N-acetyl-α-D-glucopyranoside | α-1 | – | 0 | – |
| *n*-nitrophenyl-N-acetyl-β-D-glucopyranoside | β-1 | – | 0 | – |
| *n*-nitrophenyl-N-acetyl-β-D-galactopyranoside | β-1 | – | 0 | – |
| *n*-nitrophenyl-β-D-glucuronide | β-1 | – | 0 | – |
| *n*-nitrophenyl-α-D-glucopyranoside | α-1 | – | 0 | – |
| *n*-nitrophenyl-α-D-fucopyranoside | α-1 | – | 0 | – |
| *n*-nitrophenyl-β-D-xylopyranoside | β-1 | – | 0 | – |
| *n*-nitrophenyl-α-D-mannopyranoside | α-1 | – | 0 | – |
| Naringin | α-1.2 | 0.77 | 36.0 | 46.8 |
| Neohesperidin | α-1.2 | 3.3 | 10.0 | 3.1 |

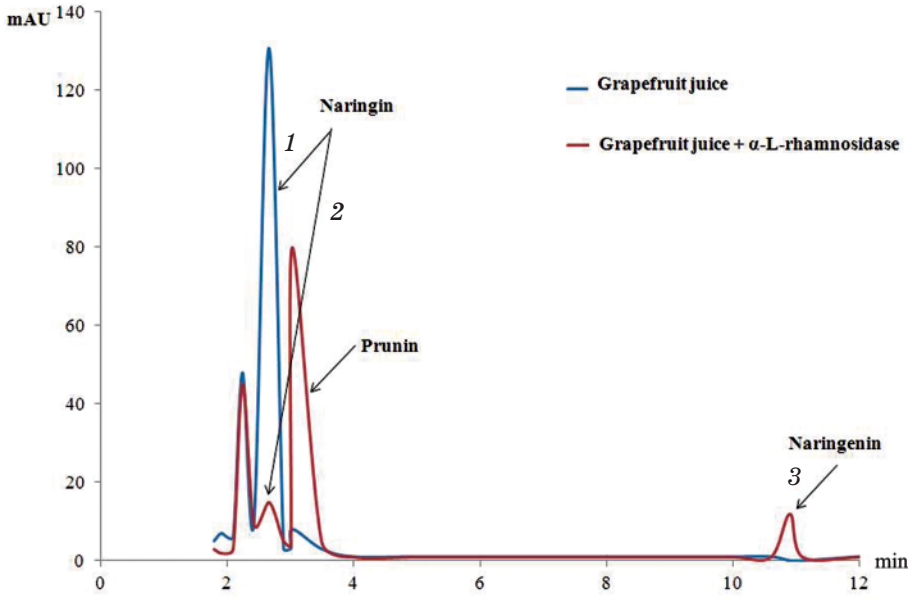


*Fig. 1.* **Naringin hydrolysis in grapefruit juice by the action of *C. albidus* α-L-rhamnosidase (40 °C, 60 min), determined by HPLC:**
naringin concentration: *1* — 255 µg/ml; *2* — < 5 µg/ml; naringenin concentration *3* — 14 µg/ml

*C. albidus* actively hydrolyzed narirutin, naringin and hesperidin in orange juice (Fig. 3), similarly to α-L- rhamnosidase of *Aspergillus aculeatus* [9]. We also noted lower amount of rutin in green tea after enzyme treatment (Fig. 4).

Thus, by specificity of action and the ability to cleave off α-bound rhamnose of natural and synthetic substrates, α-L-rhamnosidase of *C. albidus* is close to α-L-rhamnosidases and naringinases of *Penicillium decumbens*, *Aspergillus niger*,

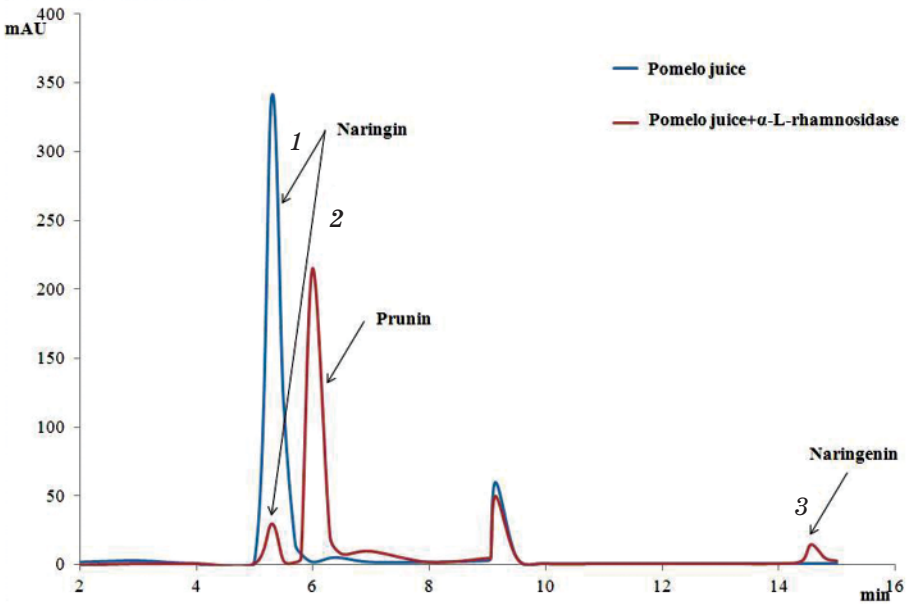


*Fig. 2.* **Naringin hydrolysis in pomelo juice by the *C. albidus* α-L-rhamnosidase (40 °C, 60 min), determined by HPLC:**
naringin concentration: *1* — 915 μg/ml; *2* — < 60 μg/ml; naringenin concentration: *3* — 24 μg/ml

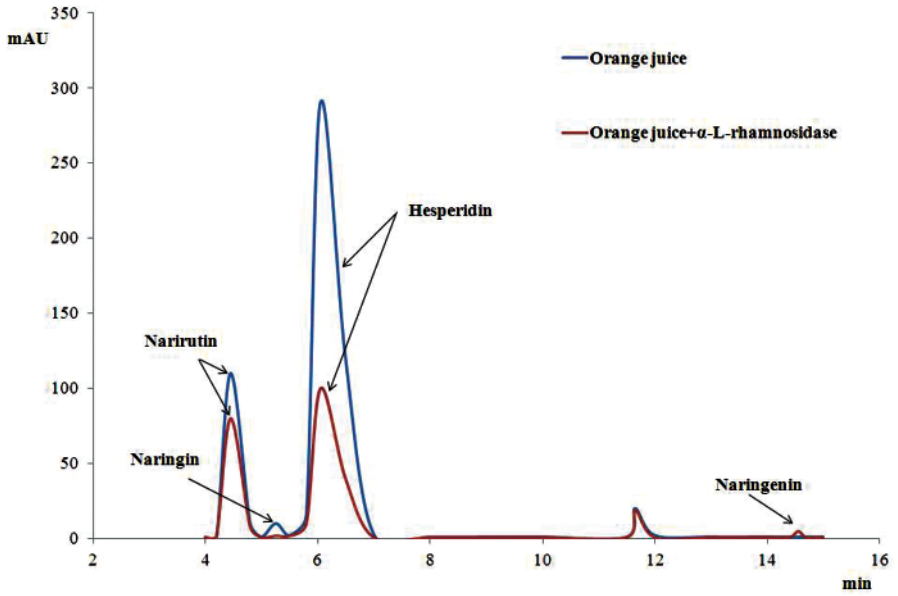


*Fig. 3.* **Concentration of naringin, naringenin, hesperidin and narirutin in orange juice before and after *C. albidus* α-L-rhamnosidase treatment:**
40 °C, 60 min

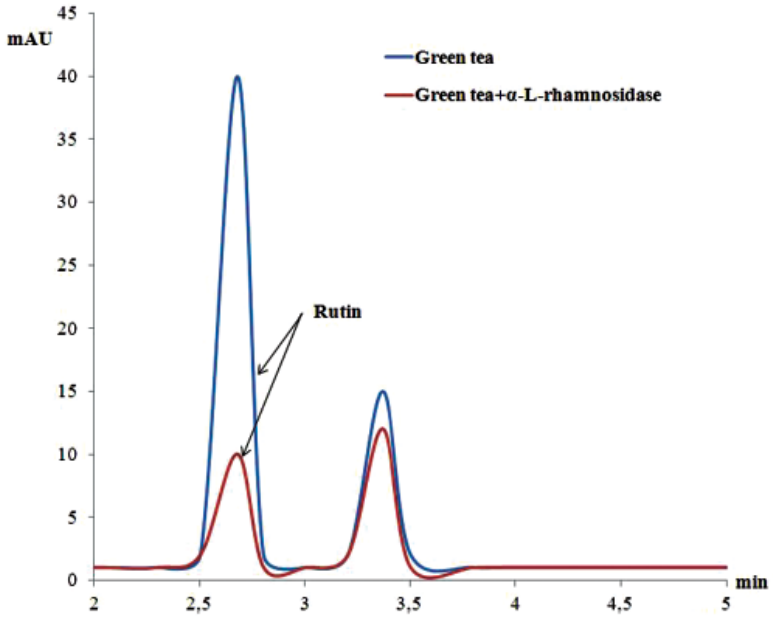


*Fig. 4.* **Rutin concentration in green tea before and after treatment with *C. albidus* α-L-rhamnosidase:** 40 °C, 60 min, HPLC

*Aspergillus aculeatus*, *Cryptococcus laurentii* [20], but its activity is 1.5–2 times higher. We also showed that α-L-rhamnosidase of *C. albidus* cleaved the naringin of citrus juices into prunin and naringenin relatively fast. The glycoside removal efficiency was 94 and 98%, respectively. The enzyme showed ability to hydrolyze naringin, neohesperidin, narirutin, hesperidin and rutin, evidence of the high potential of *C. albidus* α-L-rhamnosidase for use in the industries of juice production and to obtain biologically active flavonoids.

## REFERENCES


1. *Ribeiro I. A., Ribeiro M. H. L.* Naringing and naringenin determination and control in grapefruit juice by a validated HPLC method. *Food Control*. 2008, 19 (4), 432–438. https://doi.org/10.1016/j.foodcont.2007.05.007.
2. *Monti D., Pisvejcova A., Kren V., Lama M., Riva S.* Generation of an α-L-rhamnosidase library and its application for the selective derhamnosylation of natural products. *Biotechnol. Bioeng*. 2004, 87 (6), 763–771.
3. *Quideau S., Deffieu D., Douat-Casassus C., Pouysegu L.* Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew. Chem. Int. Edit*. 2011, 50 (3), 586–621. https://doi.org/10.1002/anie.201000044
4. *Xiao J.* Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Crit. Rev. Food Sci. Nutr.* 2017, 57 (9), 1874–1905. https://doi.org/10.1080/10408398.2015.1032400
5. *Walle T.* Absorption and metabolism of flavonoids. *Free Radic. Biol. Med*. 2004, 36 (7), 829–837.
6. *Valentova K., Vrba J., Bancirova M., Ulrichova J., Kren V.* Isoquercitrin: pharmacology, toxicology, and metabolism. *Food Chem. Toxicol.* 2014, 68, 267–282. https://doi.org/10.1016/j.fct.2014.03.018
7. *Slamova K., Kapesova J., Valentova K.* "Sweet Flavonoids": Glycosidase-catalyzed modifications. *Int. J. Mol. Sci.* 2018, 19 (7), 2126. https://doi.org/10.3390/ijms19072126
8. *Weignerova L., Marhol P., Gerstorferova D., Kren V.* Preparatory production of quercetin-3-β-D-glucopyranoside using alkali-tolerant thermostable α-L-rhamnosidase from *Aspergillus terreus*. *Bioresour. Technol.* 2012, 115, 222–227. https://doi.org/10.1016/j.biortech.2011.08.029
9. *Gonzalez-Barrio R., Trindade L. M., Manzanares P., de Graaff L. H., Tomas-Barberan F. A., Espin J. C.* Production of bioavailable flavonoid glucosides in fruit juices and green tea by use of fungal alpha-L-rhamnosidases. *J. Agric. Food Chem.* 2004, 52 (20), 6136–6142. https://doi.org/10.1021/jf0490807
10. *Ni H., Xiao A. F., Cai H. N., Chen F., You Q., Lu Y. Z.* Purification and characterization of *Aspergillus niger* α-L-rhamnosidase for the biotransformation of naringin to prunin. *Afr. J. Microbiol. Res.* 2012, 6 (24), 5276–5284. https://doi.org/10.5897/AJMR12.1229
11. *Yadav S., Yadava S., Yadav K. D.* α-L-Rhamnosidase selective for rutin to isoquercitrin transformation from *Penicillium griseoroseum* MTCC-9224. *Bioorg. Chem.* 2017, 70, 222–228. https://doi.org/10.1016/j.bioorg. 2017.01.002
12. *Mueller M., Zartl B., Schleritzko A., Stenzl M., Viernstein H., Unger F. M.* Rhamnosidase activity of selected probiotics and their ability to hydrolyse flavonoid rhamnoglucosides. *Bioproc. Biosyst. Eng.* 2018, 41 (2), 221–228. https://doi.org/10.1007/s00449-017-1860-5
13. *Amaretti A., Raimondi S., Leonardi A., Quartieri A., Rossi M.* Hydrolysis of the rutinose-conjugates flavonoids rutin and hesperidin by the gut microbiota and bifidobacteria. *Nutrients*. 2015, 7 (4), 2788–2800. https://doi.org/10.3390/ nu7042788
14. *Zhu Y., Jia H., Xi M., Xu L., Wu S., Li X.* Purification and characterization of a

naringinase from a newly isolated strain of *Bacillus amyloliquefaciens* 11568 suitable for the transformation of flavonoids. *Food Chem.* 2017, 214, 39–46. https://doi.org/10.1016/j.foodchem.2016.06.108
15. *Singh P., Sahota P. P., Singh R. K.* Evaluation and characterization of new α-L-rhamnosidase-producing yeast strains. *J. Gen. Appl.Microbiol.* 2015, 61 (5), 149–156. https://doi.org/10.2323/jgam.61.149
16. *Gudzenko O. V., Varbanets L. D.* Purification and and physico-chemical properties of α-L-rhamnosidase *Cryptococcus albidus*. *Microbiol. Zh.* 2012, 74 (6), 16–23. (In Russian).
17. *Chaplin M.E., Kennedy J.E.* Carbohydrate analysis. *Oxford: Washington: IRL Press.* 1986, 228 p.
18. *Davis D. W.* Determination of flavonones in citrus juice. *Anal. Biochem.* 1947, 19, 476–478.
19. *Dixon M., Webb E.* Enzymes. *Moskva: Mir.* 1982, 1, 98–109. (In Russian).
20. *Yadav V., Yadav P. K., Yadav S., Yadav K. D. S.* Alpha-L-rhamnosidase: a review. *Proc. Biochem.* 2010, 45 (8), 1226–1235. https://doi.org/10.1016/j.procbio. 2010.05.025

## ДЕГРАДАЦІЯ ФЛАВОНОЇДІВ α-L-РАМНОЗИДАЗОЮ *Cryptococcus albidus*

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Метою роботи було дослідити можливості практичного застосування субстратної специфічності α-L-рамнозидази *Cryptococcus albidus*. Для визначення активності та специфічності дії ензиму використовували *n*-нітрофенільні похідні моносахаридів. Здатність гідролізувати природні субстрати оцінювали методами Davis і високоефективної рідинної хроматографії. Встановлено, що ензим виявляє вузьку специфічність щодо глікону синтетичних субстратів, гідролізує тільки *n*-нітрофеніл-α-L-рамнопіранозид ($К_m$ 4,5 мМ) та *n*-нітрофеніл-β-D-глюкопіранозид ($К_m$ 10 мМ). Найефективніше α-L-рамнозидаза *C. albidus* деградувала нарингін ($К_m$ 0,77 мМ), вивільнюючи прунін та нарингенін. $К_m$ для неогесперидину дорівнювала 3,3 мМ. Ефективність гідролізу нарингіну грейпфрутового та помелового соку становила 94 та 98% за 60 хв (40 °С, 2 од/мл). У результаті оброблення зеленого чаю та апельсинового соку відзначалося зменшення вмісту рутину, нарирутину та гесперидину, що свідчить про здатність α-L-рамнозидази відщеплювати α-1,2- та α-1,6-зв'язану рамнозу від природних флавоноїдів. Таким чином, показано ефективність використання α-L-рамнозидази *C. albidus* для гідролізу флавоноїдів цитрусових соків та зеленого чаю з метою поліпшення їхніх смакових властивостей та отримання біодоступних глюкозидів флавоноїдів.

***Ключові слова:*** *Cryptococcus albidus*, α-L-рамнозидаза, нарингін, неогесперидин, рутин, флавоноїди, цитрусові соки, зелений чай.

## ДЕГРАДАЦИЯ ФЛАВОНОИДОВ α-L-РАМНОЗИДАЗОЙ *Cryptococcus albidus*

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Целью роботы было исследовать возможности практического применения субстратной специфичности α-L-рамнозидазы *Cryptococcus albidus*. Для определения активности и специфичности действия энзима использовали *n*-нитрофенильные производные моносахаридов. Способность гидролизовать природные субстраты оценивали методами Davis и высокоэффективной жидкостной хроматографии. Установлено, что энзим проявляет узкую специфичность относительно гликона синтетических субстратов и гидролизует только *n*-нитрофенил-α-L-рамнопиранозид ($К_m$ 4,5 мМ) и *n*-нитрофенил-β-D-глюкопиранозид ($К_m$ 10 мМ). Наиболее активно α-L-рамнозидаза *C. albidus* деградирует нарингин ($К_m$ 0,77 мМ), высвобождая прунин и нарингенин. $К_m$ для неогесперидина составила 3,3 мМ. Эффективность гидролиза нарингина в грейпфрутовом и помеловом соке составила 94 и 98% за 60 мин (40 °С, 2 ед/мл). В результате обработки α-L-рамнозидазой зеленого чая и апельсинового сока отмечалось снижение содержания рутина, нарирутина и гесперидина, что свидетельствует о способности отщеплять α-1,2- и α-1,6-связанную рамнозу от природных флавоноидов. Таким образом, показана эффективность использования α-L-рамнозидазы *C. albidus* для обработки цитрусовых соков и зеленого чая с целью улучшения их вкусовых качеств и получения биодоступных глюкозидов флавоноидов.

***Ключевые слова:*** *Cryptococcus albidus*, α-L-рамнозидаза, нарингин, неогесперидин, рутин, флавоноиды, цитрусовые соки, зеленый чай.