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NEW MODE OF ENDOINULASE (EC 3.2.1.7) PURIFICATION

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At the first time the mode of endoinulase purification from dapple has been proposed. Endoinulase was released from *Taraxacum officinale* root cells, solved in water, settled at pH 6.2, then the sediment was treated by buthanol. The purification was obtained by gel-filtration method applying organic solvents (buthanol and acetone) as well as a column containing G-150 Sephadex (with 2.0×120 cm^2 sizes). The enzyme was purified 71.3 times, its molecular mass was 176 *kDa*.

***Keywords*:** inulin, endoinulase, fructose, glucose.

Introduction. Significance of inulin (inulin polysaccharide is mainly consisted of digestible mono-sugar fructose, the amount of which according to obtained data is 90%) in food quality and value enhancing area is mentioned repeatedly [1–5]. The author gives data concerning to inulin effect in intestines, particularly in colon, where it effects on digestion process, as well as exchange processes of proteins, carbohydrates and fat. Besides it is shown that inulin supports the growth of microorganisms in intestines, decreases the possibility of intestine polyp formation and cancer. Inulin decreases the amount of sugar and cholesterol in blood as well [6].

Inulase enzyme cleaves the inulin and is appeared in 2 modes: exoinulase (EC 3.2.1.26; *β-d*-fructophuranosidase) and endoinulase (EC 3.2.1.7; *β*-fructanohydrolase). Inulins obtained from different plants have fructose rings with different lengths that end by *α-d*-glucophuranosil molecule. Inulin and its derivatives are used in food industry, the production of which is permanently increased. The main goal is directed to improve food quality. This question is intensively discussed in [2].

Inulase enzyme is being obtained by many authors from different microorganisms. They are obtained especially from water extracts released from microorganism pure cultures [6–16].

The aim of present paper is to obtain biological, biochemical and physicochemical properties of the pure enzymatic preparation endoinulase.

Materials and Methods. As an investigation object dandelion (*Taraxacum officinale*) was used, which is gathered from meadows. This plant grows in the territory of Armenia everywhere, especially in mount zones. According to our investigations the dandelion growing in meadows possesses high enzymatic

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(inulase) activity [17]. In wild (gardens, meadows, edges of highways) inulase gets to high activity during herb bud formation and flowering [17, 18].

The reactives used for enzyme purification were pure from analytical point of view. To obtain inulase enzyme preparation, the gathered roots of dandelion from meadows were eluted by distilled water, peel of root was removed, dried in room conditions, pestled and mashed in mortar until receiving of homogeneous mass. Acetone is added, mixed and centrifuged in cold conditions with 10000 *g* 15 *min*. The sediment is separated from the supernatant. For further experiments the supernatant was used.

pH optimization is made under different values of pH using different buffer solutions that have pH borders, for example, 0.1 *M* sodium acetate buffer (4.0–5.5), sodium phosphate buffer (6.5–8.0), tris-HCl (8.0–9.0), pH was measured by pH-meter.

Intercellular inulase enzyme obtained from dandelion (*Taraxacum officinale*) was purified by hel-filtration method [19] using Sephadex G-150 (2.0×120 cm^2), for enzyme sedimentation buthanol and acetone solvents with different densities were used.

For purified enzyme mass (molecular mass) determination marker proteins ("Sigma") were used and unknown mass (x) is found by relative mobility [16]. For inulase molecular mass determination active fractions were used via proteins with known molecular masses, *Da*: fibrinogene (33000), catalase (24000), aldolase (16000), albumin (45000) and cytochrome C (12300).

Hel-filtration is widely used for molecular mass determination of polymers, especially proteins. But following literature data it may be concluded that enzyme molecular mass is changed depending on applied method and conditions, when relationships between enzymatic protein monomer forms may change.

High molecular mixtures effect on the results of molecular mass of enzymes. That is why the stable results are obtained only using preparations having low density and high purification.

Results and Discussion. Purification results of inulase enzyme obtained from dandelion are presented in Table 1.

Table 1

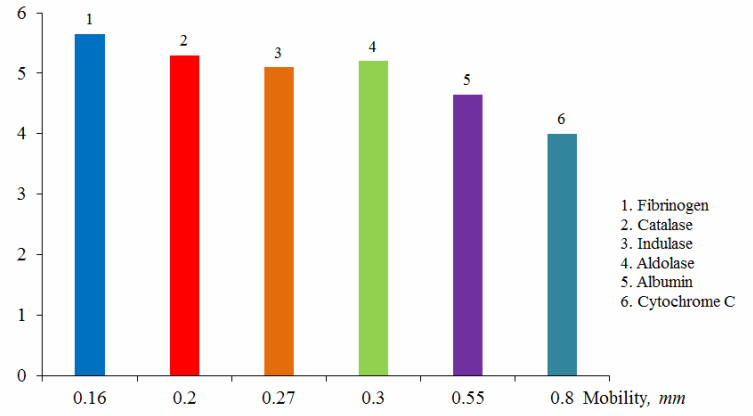
Inulase enzyme purification process of dandelion (Taraxacum officinale Wigg)

Fractions	Volume, *mL*	Enzyme concentration unit/*mL*	Output	Purification degree
initial extract	12000	205.00	100	1.00
sedimentation pH 6.2	5000	216.00	44	2.47
treatment by buthanol	4450	166.00	30	8.30
sedimentation by 60% cold acetone	55	2623.00	28	12.42
sedimentation by 45% cold acetone	20	3324.41	21	28.40
sedimentation by 40% cold acetone	20	69120.00	15	71.35

It is obvious from Tab. 1, that at pH 6.2 after one day incubation and 8000*g* and 20 *min* centrifugation the enzyme is purified almost 2.5 times, but after

buthanol treatment 8.8 times purified preparation is obtained. In the next step enzyme purification is made by cold acetone since in acetone incubated in room temperature inulase enzyme specific activity is a little decreased. Continuing inulase purification process in the next steps 71.3 times purified inulase with 15% output has been obtained by cold acetone.

Inulase Molecular Mass Determination Process. Inulase molecular mass determination diagram is presented on Figure. The obtained results show that this method justifies our approach for molecular mass determination.



Inulase molecular mass determination diagram.

According to protein mobility presented on the Figure, inulase molecular mass was determined, that was 176.5 *kDa*.

The Optimum of Inulase Enzyme pH of Dandelion. The results of inulase enzyme functioning pH optimum are presented in Tab. 2. It is obvious from Tab. 2, that pH optimum of inulase enzyme is 6.2. If at pH 6.2 there are 362.0 and 1.3 *mM* criteria of fructose and glucose, at 5.4 and 7.4 pH values there are 300 *mM* fructose and 233 *mM* fructose respectively.

Table 2

Inulase enzyme pH optimum of dandelion

pH value	Inulin, %	Fructose, *mM*	Glucose, *mM*	Fructose in extract, %	Glucose in extract, %
5.4	10.7	300.0	2.2	99.3	0.7
6.2	–	362.0	1.3	99.6	0.4
6.6	–	263.0	1.6	96.4	0.6
7.0	–	286.0	3.3	98.8	1.2
7.4	–	233.0	2.4	99.0	1.0

The fact is interesting that at hydrolysis by dandelion inulase fructose percentage versus glucose is high from 99% that is an enough high criterion and is good for syrup production, since glucose amount in the mixture is in 0.4–1.2% range, it is considered a good criterion, hence, an enzyme obtained from dapple has an advantage. Dandelion comprises an inulin, which is an additional source of fructose and glucose. In literature we did not notice an enzyme having similar properties from different groups of microorganism [5, 6, 20].

Conclusion. The purification of inulase enzyme (endoinulase) in natural conditions (from dandelion growing in meadows) by Sephadex G-150, applying gel-filtration was presented. Enzyme settlement by buthanol and cold acetone was carried out, which makes possible to obtain 71.3 times purified inulase with 15% outcome. The enzyme cleaves inulin and gives fructose with higher than 99% criteria and 0.4% glucose containing juice (syrup). These criteria indicate that it is advisable to apply enzymatic preparation with industrial goals, as well as to obtain fructose-glucose juice with 99% and more percentage.

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REFERENCES

1. **Roberfroid M.B.** Dietary Fiber, Inulin and Oligofructose: a Review Comparing Their Physiological Effects. // CRC Crit. Rev. Food Sci. Nutr., 1993, v. 33, p. 103–148.
2. **Roberfroid M.B.** Prebiotics and Synbiotics: Concepts and Nutritional Properties. // Br. J. Nutr., 1998, v. 80, p. S197–S202.
3. **Roberfroid M.B.** Functional Foods: Concepts and Application To Inulin and Oligofructose. // Br. J. Nutr., 2002, v. 87, № 2, p. S139–S143.
4. **Roberfroid M.B.** Inulin-Type Fructans as Functional Food Ingredients. Boca Raton, FL: CRC Press, 2005.
5. **Roberfroid M.B.** Prebiotics: the Concept Revisited. // J. Nutr., 2007, v. 37, p. 830–837.
6. **Kholyavka M.G.** About Possible Application of Heterogeneous Preparations of Inulase for Obtaining of Functional Nutrition Products. Voronej State University, 2010 (in Russian).
7. **Brevnova E.E., Kozlov D.J., Efremov B.D., Benevolensky S.V.** Inulase-Secreting Strain of Saccharomyces Cerevisiae Produces Fructose. // BiotechnolBioeng., 1998, v. 60, p. 492–497.
8. **Uhm T.B., Chung M.S., Lee S.H., Gourrons F., Housen J., Kim J.H., Van Beeumen J., Hage B., Vandenhaute J.** Purification and Characterization of Aspergillus Ficuum Endoinulinase. // Biosci. Biotechnol. Biochem., 1999, v. 63, p. 146–151.
9. **Baumgartners S., Dax T.G., Praznik W., Falk H.** Characterization of the Highmolecular Weight Fructan Isolated from Garlic (*Allium sativum* L.). // Carbohydr. Res., 2000, v. 328, p. 117–183.
10. **Wang J.H., Liu Y.Y., Wany Y.R., Sheng Wu Gong, Cheng Xue Bao** A Study on Screening and High Density Cell Cultivation of a Yeast Strain Kluyveromyces with High Inulinase Yielding and Its Enzymology Properties. // Chinese Journal of Biotechnology, 2000, v. 16, p. 60–64.
11. **Hamdy H.S.** Purification and Some Important Characters of Extracellular Inulinase of Alternaria Alternata (Fr.) Keissler. // Indian J. Exp. Biol., 2002, v. 40, p. 1393–1398.
12. **Kwon H.J., Jeon S.J., You D.J., Kim K.H., Jeony Y.K., Kim Y.M., Kim B.M.** Cloning and Characterization of an Exoinulinase from *Bacillus polymyxa*. // Biotechnol. Lett., 2003, v. 25, p. 155–159.
13. **Skowronek M., Fiedurek J.** Selection of Biochemical Mutants of *Aspergillus niger* Resistant to Some Abiotic Stresses with Increased Inulinase Production. // J. Appl. Microbiol., 2003, v. 95, p. 686–692.
14. **Jing W., Zhengyu J., Bo J., Xueming X.** Separation and Identification of Exo- and Endoinulinase from *Aspergillus ficuum*. // Curr. Microbiol., 2003, v. 47, p. 109–112.
15. **Gill P.K., Manhas R.K., Singh I., Singh P.** Purification and Characterization of an Exo-inulinase from *Aspergillus fumigatus*. // Appl. Biochem. Biotechnol., 2004, v. 117, p. 19–32.
16. **Bender I.P., Mazutti M.A., Oliveira D., Lucco M., Treichel H.** Inulinase Production by Kluyveromyces Marxianus NRRL Y-7571 Using Solid State Fermentation. // Appl. Biochem. Biotechnol., 2006, v. 129, № 132, p. 951–958.
17. **Baghdasaryan Ye.G., Telunts A.G., Baghdasaryan G.Ye.** Gavar State University International Conference Book, 2012, v. 14, p. 87–91 (in Armenian).
18. **Baghdasaryan Ye.G., Telunts A.G.** Goris State University International Conference Book, 2010, p. 72–77 (in Armenian).
19. **Dikson M., Uebb E.** Enzymes. M.: Mir, 1966 (in Russian).
20. **Park J.P., Bae J.T., You D.J., Kim B.W., Yun J.W.** Production of Inulooligosaccharides from Inulin by a Novel Endoinulinase from *Xanthomonas* sp. // Biotechnology Letters, 1999, v. 19, p. 1043–1046.