Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

RESEARCHES CONCERNING THE ENZYMATIC ACTION OF BYPRODUCT GRAPES

Mihaela DUMITRU[1,2], Ștefana JURCOANE[2,3]

[1]National Research Development Institute for Biology and Animal Nutrition (IBNA), 1 Calea București, Balotești, Ilfov, 077015, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd., District 1, Bucharest, Romania
[3]Academy of Romanian Scientists Bucharest, Romania

Corresponding author email: mihaela.dumitru@ibna.ro

Abstract

The objective of the present research was to convert the grape pomace as energy source, by controlling to increase hydrolysis of the complex sugars (celluloses, hemicelluloses) to fermentable sugars. Hydrolysis can be performed chemically or enzymatically following the appropriate pretreatment steps. The chemical pretreatment of the grape pomace substrat present many disadvantages such as the high costs, the special conditions and the formation of toxic by-products. For this reason, we used hydrolysis enzymatic process, as the most efficient method. The efficiency a commercial enzymatic product - MethaPlus L 100 (containing β-glucanase, cellulase, xylanase) was investigated to hydrolyse dry grape pomace, after physical pretreatment (ground 1mm sieve diameter) to fermentable sugar. The enzyme MethaPlus L 100 was added in different concentrations 0.1 – 1 % (w/w) referred to the substrate dry matter content. The reducing sugars concentration increased of 4.0 fold (when 0.01% MethaPlus L 100 was added) reported relatively to the untreated sample. However, an increase of enzymes concentrations not economically relevant. In the future, the efforts should be focused on the improving the enzymatic treatment of the grape pomace, to be used as nutritional component in animal feed.

Keywords*: enzymes, dry grape pomace, enzymatic hydrolysis, reducing sugars.*

INTRODUCTION

Continued growth in the consumption of animal products (meat, milk, eggs) livestock research oriented towards finding ways of meeting the requirements of the market. Also, it has been sought to solve problems relating to nutrition and health of animals, increase the bioavailability of nutrients present in the feed young animals, whose enzyme is not fully developed equipment, reducing the level of removal of nutrients by manures.

The addition of exogenous enzyme preparation in animal feed, presents the capacity to degrade various nutrients in the environment, carbohydrates, lipids, proteins, that turn them into simple compounds, absorbable, which penetrates the cell membrane (Beckers and Théwis, 2004).

Lignocellulosic biomass are mainly composed of cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) (Badger et al., 2000; Girio et al., 2010; Mielenz, 2001; Pettersen, 1984).

Cellulose is a homopolysaccharide composed of β-D-pyranose units, linked by β-1, 4- glycosidic bonds. Cellobiose is the smallest repetitive unit and it is formed by two glucose monomers. The long-chain cellulose polymers are packed together into microfibrils by hydrogen and Van der Waals bonds (Vijai et al., 2014).

Hemicellulose is a mixture of polysaccharides, including pentoses, hexoses and uronic acids (McMillan, 1993). Hemicelluloses constitution enters into the plant cell membrane and that may not be degraded by endogenous enzymes, the animal is deprived of the use of plant cell intracellular constituents. For these reasons, hemicelluloses are classified as anti-nutritional factors for monogastric animals (Israel Roming et al., 2010).

The dried grape pomace (DGP) is a by-product of vine-making from grapes, which can be used in animal feeding mainly due to its properties of natural antioxidant and also to its valuable content of fatty acids, energy and protein (Habeanu et al., 2015).

Studies have been performed on the antioxidant effects of grape extracts due to their high content in polyphenols (Brenes et al. 2008, Chedea et al. 2010) the focus being on the evaluation of the effectiveness of these raw materials on animal products quality (Rojas and Brewer, 2008).

Actually, grape pomace is a blend of separated solids must (marc) or wine (fermented pomace) made of peels (55-65%), seeds (18.25%) and traces of grape clusters and wine extracted from pressing (Pomohaci et al., 2000).

Also, the grape pomace can be a potential source of fermentable sugars, to be used in animal feed.

The resulting product, after obtaining wine or other alcoholic fermentation of fruit, is a liquid condition and to be placed in animal nutrition requires preliminary drying (Voicu et al., 2014).

Grape pomace (GP) consists of the four major poly-saccharides in nature, namely cellulose (consisting of glucose subunits), hemicellulose (glucose, mannose, xylan and arabinose subunits), starch (glucose subunits) and pectin (D-galacturonic acid subunits) (Hulme, 1970, cited by Korkie et al., 2002).

The hydrolyzis can be performed chemically or enzymatically, following the appropriate pretreatment steps.

The hydrolysis processes used in the past, were essentially chemical, but the costs and the formation of toxix by-products made them noncompetitive (Diguta et al., 2007; Israel Roming et al., 2005).

Enzymatic processes, which hold several advanges, are now substituting the chemical ones. The efficiency of enzymatic process is quite hite and the mild process conditions require neither expensive materials nor hight process energy (Diguta et al., 2007).

The use of enzymatic complex (β-glucanase, cellulase, xylanase) for hydrolysis of lignocellulosic substrate (grape pomace) into fermentable sugars gains more and more interest.

In this study, the objective was to examine the capacity of enzymatic hydrolysis of dry grape pomace, by using different concentrations of MethaPlus L 100 enzyme product. The optimum level of exogenous enzyme added on our substrate was done as well.

MATERIALS AND METHODS

Substrate

The by-product used in our study was grape pomace, provided by a Romanian distillery (SC Dionis Agrifood, in collaboration with the National Research Development Institute for Biology and Animal Nutrition (IBNA) - Balotești, Romania). The wet GP was dried at 90°C in a counter-flow hot air conveyor dryer (Habeanu et al., 2015).

This mixture was milled using us strainers 1 mm, to increase the accesibility of hydrolitic enzymes at the complex polysaccharides (celluloses, hemicelluloses, starch).

Enzyme preparation

Enzymatic hydrolysis was performed with a commercial enzyme in different concentrations 0.1-1% w/w referred to the substrate dry matter content. The commercial enzyme preparation used is: MethaPlus L 100 (β-glucanase, cellulase, xylanase) produced by BIOPRACT GmbH, Germany.

Enzymatic hydrolysis

The enzymatic treatment was performed in one step process, by using MethaPlus L 100, for an additional hydrolysis period of 20 h. The hydrolysis incubation took place at 55°C, pH=5-5.5, on a rotary shaker as 200 rpm. The experiment were performed in duplicate and the results are presented as mean values. For each trial, were prepared a control sample (without enzyme additions).

Determination of reducing sugar concentration

After conducting the process of hydrolysis (20 hours), all the samples were centrifuged at 5000 rpm, time to 5 minutes.

Reducing sugars were determined in superntant by using dinitrosalycilic acid reagent (DNS) at optical 640 nm, by the modified method described by Petterson and Porath (Iordăchescu and Dumitru, 1980). The degree of recalcitrant molecules (celluloses, hemicelluloses) was estimated by quantifying the amount of reducing sugars formed during enzymatic hydrolysis.

RESULTS AND DISUSSIONS

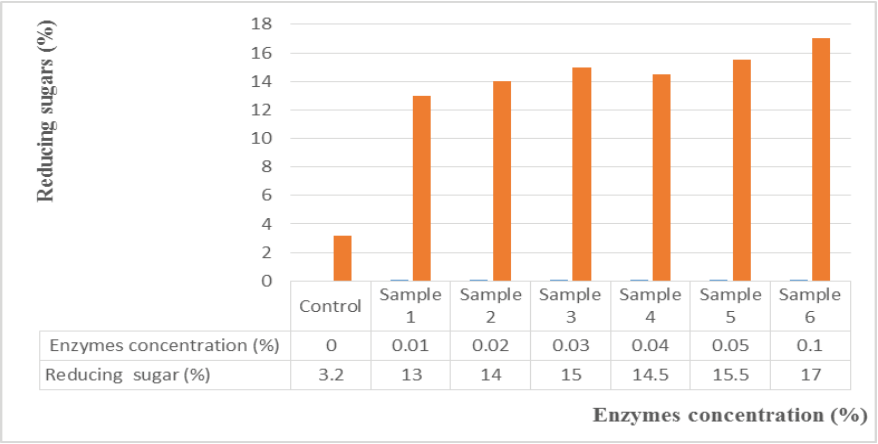
The grape pomace can be used as nutritional component in animal feed due the containing of

polysaccharides (Hulme, 1970, cited by Korkie et al., 2002) and high amounts of polyphenols with antioxidant properties (Alonso et al., 2002).

The present research was focused to convert the grape pomace as energy source, by enzymatic hydrolysis of the complex sugars (celluloses, hemicelluloses) to fermentable sugars (Figure 1). The enzymatic treatment was performed by using MethaPlus L 100 in different concentrations (Figure 1).

The reducing sugars concentration in the control (whithout enzymes addition) is lower, aproximately 5 times, compared with the last sample, where the concentration was hight.

According to figure 1, the reducing sugars was 13% by using 0.01% MethaPlus L 100, representing an improving of 4 fold, reported relatively to the untreated sample. Additionally, an increase of enzymes concentration from 0.01 to 0.1% enhanced the reducing sugars concentration with approximately 24%. However, in economic terms, the increase of enzymes concentration is not relevant, because between the level concentrations of reducing sugars were not signifficant differences.



	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Enzymes concentration (%)	0	0.01	0.02	0.03	0.04	0.05	0.1
Reducing sugar (%)	3.2	13	14	15	14.5	15.5	17

Figure 1. Reducing sugars of grape pomace after enzymatic hydrolysis

CONCLUSIONS

The object to this research was to investigate the influence a commercial enzymatic product at different concentrations, on the conversions of energy crops to fermentable sugars, in our case, MethaPlus L100 (enzymatic complex), by using as substrate dry grape pomace.

The optim level were obtained at the 0.01% concentration of enzyme product in the grape pomace substrate.

In the future, the efforts shoulds be focused on the improving the enzymatic treatment, by using other enzymes in association with/or not MethaPlus L100.

ACKNOWLEDGMENTS

This research has been financially supported by Romanian National Program ADER (No 3.3.4/2015).

REFERENCES

Alonso A., Guillean D., Barroso C., Puertas B., Garcia, A., (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. Journal of Agricultural and Food Chemistry, 50, 5832–5836.

Badger PC. In: Jannick J, Whipsekey A, (2000). Trends in new crops and new uses. Alexandria, VA: ASHS Press. p. 17 - 21.

Beckers Y., Théwis A., (2004). Influence sur les performances zootechniques de quatre xylanases ajoutées à un régime riche en froment chez le poulet de chair. Rapport final sur la prestation de

service entre l'Unité de Zootechnie de la Faculté universitaire des Sciences agronomiques à Gembloux et la société Beldem s.a., 10 p.

Chedea V S., Braicu and Socaciu C 2010. Antioxidant/prooxidant activity of a polyphenolic grape seed extract. Food Chemistry 12: 132–139.

Diguță C., Jurcoane S., Israel-Roming F., Brule M., Mukengele M., Lemmer A., Oechsner H., (2007). Studies concerning enzymatic hydrolysis of energy crops. Roumanian Biotechnological Letters, Vol. 12, No. 2, p. 3203-3207.

Girio F. M., Fonseca C., Florbela C., Duarte LC., Marques S., Bogel-Lukasic R., (2010). Hemicellulose for fuel ethanol. Bioresour Technology, 101: 4775-800, available at ScienceDirect.

Harmsen P. F. H., Huijen W. J. J., Bermúdez López L. M. and Bakker R. R. C., (2010). Literature Review of Physical and chemical Pretreatment Processes for Lignocellulosic Biomass. Wageningen University & Food and Biobased Research, pp. 1-54, ISBN 9789085857570.

Mihaela Habeanu, Nicoleta A. Lefter, Mariana Ropota, Veronica S. Chedea, Anca Gheorghe, Smaranda M. Toma, Georgeta Ciurescu, Cătălin Dragomir. 2015. *Dried grape pomace influenced fatty acids composition of Longissimus dorsi muscle and plasma polyphenols spectrum in finishing pigs*. Indian Journal of Animal Sciences.

Hulme A.C., (1970). The Biochemistry of Fruits and their Products, Vol. 1. New York Academic Press.

Israel Roming F., Câmpeanu G., Zamfir E., (2005). Improving the susceptibility of lignocellulosic materials to enzymatic hydrolysis by alkalino-peroxidicpre-treatment. Roumanian Biotechnological Letters, vol. 10, no. 6, pp. 2435-2440.

Iordăchescu D., Dumitru I.F., (1980). Practical biochemistry - proteins and enzymes, Part I, Bucharest University Press.

Knauf M., Moniruzzaman M., (2004). Lignocellulosic biomass processing: a perspective. IntSugar J;106:147 – 150.

Korkie L. J., Janse B. J. H., Viljoen – Bloom M., (2002). Utilising Grape pomace for ethanol production, vol. 23, No. 1, South Africa.

McMillan JD, (1993). Pretreatment of lignocellulosic biomass. In: Himmel ME, Baker JO, Overend RP, editors, Enzymatic conversion of biomass for fuel production. Washington, D.C: American Chemical Society; 292 - 323.

Mielenz J. R., (2001). Ethanol production from biomass: technology and commercial-ization status. Curr Opin Microbiol;4:324 – 5.

Pettersen RC., (1984). The chemical composition of wood. In: Rowell RM, editor. The chemistry of solid wood. Advances in chemistry series, vol. 207. Washington, DC: American Chemical Society; 1984. p. 115, 6.

Pomohaci N., Cioltean I., Vişan L., Rădoi F., (2002). Tuica şi rachiurile naturale, Editura Ceres, Bucureşti.

Rojas M C and Brewer M S (2008). Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *Journal of Food Quality* **31**: 173–188.

Voicu D., Voicu I., Hăbeanu M, Uță R. A., (2014). Nutreț combinat îmbogățit în acizi grași polinesaturați prin adaos de tescovină uscată de struguri, folosit în raţiile taurinelor la îngrăşat, Institutul Național de Cercetare - Dezvoltare pentru Biologie şi Nutriție Animală - IBNA Balotești, Analele IBNA vol. 30, p. 89-90.

Vijai K. G., Schmoll, Alfredo H. E., Upadhyay R. S., Druzhinina I., Tuohy M. G., (2014). Biotechology and biology of Trichoderma. British Library Elsevier, Polonia.

Ye Sun and Cheng J., (2001). Hydrolysis of lignocellulosic materials for ethanol production. Edition bioresource Technology 83, Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, NC 7695-7625, p. 1-11,USA.