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RESEARCHES CONCERNING THE LEVEL OF FERMENTABLE SUGARS FROM FEED MATERIALS IN RELATION WITH CELLULASE HYDROLYSIS BY CARBOHYDRASE ENZYME

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Abstract

The objective of this experimental study was to evaluate the enzymatic hydrolysis process in order to obtain fermentable sugars from feed materials (maize, field peas, field beans), usually used in animal nutrition. For breaking down the carbohydrates from cell materials raw, it was used a carbohydrase enzyme preparation (IUB No. 3.2.1.6) with activity of 50 FBG/g. The enzyme was tested at concentration of 0.05-0.5% (w/v). For measuring accumulation of reducing sugars, it was used 3,5 dinitrosalicylic acid (DNS) assay. Results from experimental samples supplemented with carbohydrase enzyme were compared with control sample without enzyme addition. The activity of carbohydrase enzyme was evaluated using a carboxymethyl cellulose medium (4.19 UDNS/ml). For the experimental data, the total amount of available sugars was significantly higher (P<0.0001) for peas (19.60±0.105), followed by maize (17.17±0.105) and beans field (10.50±0.055) at a concentration of 0.5% enzyme. The results suggest that hydrolytic potential of enzyme preparation to produce fermentable sugars was superior on field peas, knowed as source of protein in animal nutrition and can be used as feed additive to improve nutrient availability.

Key words: *enzymatic hydrolysis, carbohydrase enzyme, reducing sugars.*

INTRODUCTION

Lignocellulose is the major component of biomass, involves by three polymers: cellulose (35-50%), hemicelluloses (20-35%) and lignin (10-25%), for typical feedstuffs which are arranged in a complex way (Dumitru and Jurcoane, 2017; Ganna, 2012).

Lignocellulose is a high source of cheap car-bohydrates. Over the past decades, it was used as raw material for the production of a range of high value products, such as bioethanol, organic acids, enzymes and biodegradable plastics (Ravindran and Jaiswal, 2016).

The cellulose (fiber) is a major component of plant cell wall, composed of glucose molecules linked together by β-1,4 glycosidic bonds, which the pig's digestive tract is unable to unlink them and hence is degraded by microbial fermentation in the hindgut (Diguță et al., 2007, Banino, 2012).

The cellulose has been identified as the principal polysaccharide in plants with a rigid structure very difficult to decompose (Oyetunji, 2009). The basic unit of cellulose is glucose. It is the most common organic group in nature, and is one of the main energy sources for plants and animals. Cellulose's hydrolysis determines her degradation of cellulose to glucose or other simple sugars, in order to use for the production of energy, in our study, in animal nutrition (Oyetunji, 2009).

Dietary carbohydrates constitute a major fraction feed, which can be divided according to glycosidic linkages into sugars (mono and disaccharides), oligosaccharides and two broad classes of polysaccharides; starch and non-starch polysaccharides (NSP) Therefore, nutrition's swine are made up primarily of grains, along with protein supplements and other vitamins and minerals. Cereal grains make up 50% to 85% of the ingredients, which in turn provide much of the energy to the

animal (Velayudhan et al., 2015). Maize grain is among the leading cereal used in swine nutrition with a greater energy density than other cereal grains. For example, small grains, such as barley, wheat, oats, rye, and triticale form other practical ingredients in swine feeding programs.

Measurement the concentration of reducing sugars (RSs) from raw materials, can provide valuable information about the analyzed sample, for understanding the amount of sugar from feedstuffs and the activities of some enzymes which are responsible for the hydrolysis of polysaccharides (Negrulescu et al., 2012).

In this study, three types of raw materials (maize - *Zea mays* L., field peas - *Pisum sativum* L. and field beans - *Vicia faba*), used in animal nutrition, were supposed to enzymatic hydrolysis process. Investigating the efficiency of glycoside bonds, with a positive effect over polysaccharides, it was determined the level of fermentable sugars yield from different source of feed materials.

MATERIALS AND METHODS

Substrate

The raw materials (maize, field peas and field beans) used in this study, were supposed to determine the concentration of reducing sugars. The samples were provided through the National Research Development Institute for Biology and Animal Nutrition (IBNA - Baloteşti, Romania). The samples were milled to 1-3 mm, to increase the accessibility of hydrolytic enzyme to the substrate.

Enzymatic hydrolysis process of raw materials

The enzymatic treatment was performed in one step process, by using a carbohydrase enzyme preparation produced by submerged fermentation of an *Aspergillus aculeatus* microorganism with activity of 50 FBG/g (Fungal β-glucanase activity/per gram). The enzyme preparation contains endo-1,3(4)-β-glucanase, hemicellulase and polygalacturonase activities. It was used different concentrations of enzyme (0.05-0.5% w/v). The hydrolysis incubation took place at 55°C, pH=5-5.5, on a rotary shaker as 150 rpm, for 16 h. During hydrolysis process, the samples were taken and centrifuged at 5500 rpm for 20 min., to remove remaining raw materials. The experiment was performed in triplicate for each substrate and the results are presented as mean values. For all trial, were prepared a control sample (without enzyme additions) (Dumitru and Jurcoane, 2017).

Determining the reducing sugars by DNS method

It was used 3,5-dinitrosalicylic acid (DNS) (Miller, 1959) as colorimetric method, for assessing the RS extracted from various raw materials (corn, field peas, field beans), by using an carbohydrase enzyme preparation (EC/IUB 3.4.21) (Dumitru et al., 2017; Jurcoane et al., 2006).

DNS assay allows measuring the concentration of total RSs obtained as a result of enzymatic hydrolysis of polysaccharides contained in lignocellulose materials. The concentration of RSs in a sample was estimated by diluting 1 mL of the hydrolysis's liquid to 25 mL with deionized water. From diluted sample (1:25 v/v), 0.5 ml was added on 3 mL DNS reagent, supplemented with 0.5 ml buffer citrate (0.05 M, pH=4.8) and then the test tube was inserted at 100°C, for 10 min. The tubes were inserted into an ice water bath, in order to stop the reaction and to stabilize the color. The absorbance of the sample is directly proportional to the amount of RSs and was acquired at wavelength 640 nm using a Biomate 3 spectrophotometer UV-Vis.

Procedure of glucose assay

RSs can be investigated by DNS method employing glucose (0.1%) as the standard calibration curve, described by Petterson and Porath (Iordăchescu and Dumitru, 1980). DNS reacts with free carbonyl group of RSs under alkaline condition, forming 3-amino-5-nitrosalicylic acid, an aromatic compound with maximum absorption at 640 nm.

Assessing the enzymatic activity

CMCase activity was assayed follow by Petterson's and Porath's method, at 50°C, for 10 min., using 1% (w/v) CMCase (carboxymethylcellulose, medium viscosity) as substrate in the enzyme activity measurements.

One unit (U) of CMCase activity was defined as the amount of enzyme, which forms with the DNS reagent, the same optical density, similar to a milligram of glucose per minute under the assayed condition used.
The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The results were expressed as mean values ± standard deviation, the differences being considered statistically significant for P <0.05.

RESULTS AND DISCUSSIONS

All the raw material samples were hydrolyzed. The enzymatic was performed by using a carbohydrase enzyme in different concentration (0-0.5%).
For the experimental data, the total amount of available sugars was significantly higher (P<0.0001) for peas (19.60±0.105), followed by maize (17.17±0.105) and beans field (10.50±0.055) at an addition of 0.5% carbohydrase enzyme (Figure 1).The best results were obtained for field peas (19.60).

The results from ANOVA showed statistically significant differences (P<0.05), between all substrates used. Without addition of enzyme product, beans field present 4.64 RSs, compared with maize, respectively peas field, where the RSs yield are approximately similar as value.
According to Figure 1, the RSs in control maize (without enzyme addition) is lower, compared with the sample where, at an addition of 0.5% carbohydrase enzyme, the level of RSs was approximately 10 times higher.

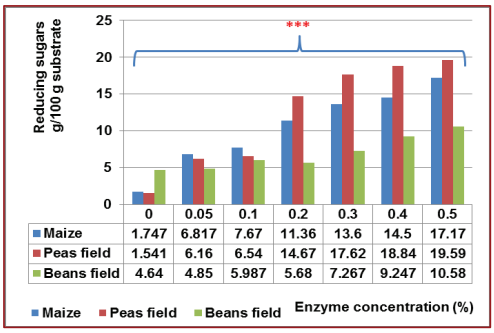
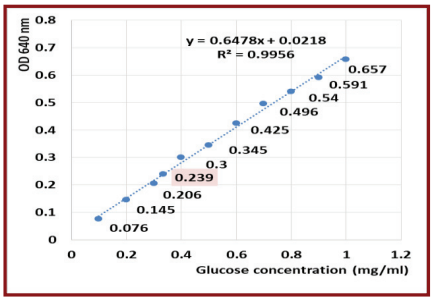


Figure 1. Reducing sugars yields for raw materials



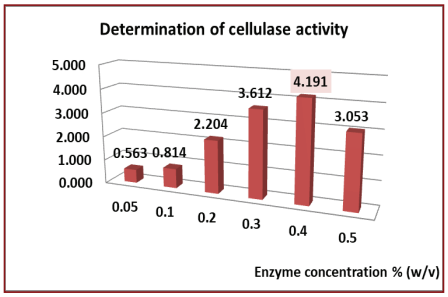


Figure 2. The enzymatic activity of carbohydrase enzyme

Figure 1 shows the variation of reducing-sugars concentration of hydrolysis liquid during the enzymatic hydrolysis. For all samples, the hydrolysis liquid did not contain sugars at the beginning, a lesser beans field, where the RSs fold registered 4.64.
The RSs increased due to the degradation of cellulose and hemicellulose at addition with enzyme. For example, the majority of feed ingredients used in monogastric animal nutrition, have in their composition carbohydrates constitute, quantitatively as the most important energy source (Banino, 2012). The small and large intestines of pigs, are both carbohydrate digestion sites and the chemical composition of carbohydrates determines if they are degraded by enzymes or microbes.
In our study, the carbohydrase enzyme preparation, at 0.4% (w/v), presents an celullase activity of 4.19 [UDNS/ml], compared with 0.5% addition, when the level of CMCase activity is lower 3.053 [UDNS/ml) (Figure 2).
The results suggest that hydrolytic potential of enzyme preparation to produce fermentable sugars was superior on field peas, followed by maize and beans field at 0.5% enzyme addition.

CONCLUSIONS

Hydrolytic potential of enzyme preparation to produce fermentable sugars was superior on field peas, knowed as source of protein in animal nutrition and can be used as feed additive to improve nutrient availability.

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