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Complementarity of the raw material composition of Very High Gravity (VHG) mashes as a method to improve efficiency of the alcoholic fermentation process

G. Kłosowski, D. Mikulski*

Kazimierz Wielki University, Department of Biotechnology, 85-671, Bydgoszcz, ul. K. J. Poniatowskiego 12, Poland

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ABSTRACT

The aim of the research was to optimize the composition of the VHG (Very High Gravity) media by selecting the proportions of various plant materials, complementary in terms of the composition of substrates affecting the fermentation process and efficiency. The addition of 25, 33 and 50% of molasses in VHG mash resulted in higher ethanol concentration above 112 g L^{-1}. The highest ethanol concentration of 129.96 ± 0.58 g L^{-1} and high actual fermentation yield as compared to theoretical one of 90.47 ± 0.40% was found for VHG mash made from all four plant materials used in the same proportions (25%). Raw spirit obtained by distillation of this medium was also characterized by the lowest content of acetaldehyde (332.8 ± 8.3 mg L^{-1} EtOH) and esters (94.3 ± 1.6 mg L^{-1} EtOH) in comparison with the spirit samples obtained from mash with a similar degree of attenuation. Addition of molasses to VHG mashes resulted also in lowering the concentration of higher alcohols in distillates to 3815 mg L^{-1} EtOH as compared to spirits obtained from substrates using starchy materials only. Use of only a complementary raw material composition ensuring that VHG mashes are rich in nutrients resulted in intensification of the alcoholic fermentation process.

1. Introduction

One of the important directions of development of fermentation technology used for the production of beverage and fuel ethanol is the introduction of VHG (Very High Gravity) technology. VHG technology uses fermentation media with a concentration of solutes higher than 250 g L^{-1} [1–3]. The use of typical raw materials usually provides satisfactory amounts and proper proportions of nutrients for yeast, but only in media of normal density (gravity), i.e. with solute concentration below 180 g L^{-1}. Along with the increase in the concentration of sugars in the mash and a higher fermentation productivity, yeast's demand for nutrients increases. These substances affect the course of the cell cycle and the mechanisms involved in the mitigation of osmotic and ethanol stress. The increased availability of amino acids, metal ions, free fatty acids and vitamins is of great importance [4–6]. In order to provide the yeast with optimal culture conditions in VHG environment and to obtain a high degree of bioconversion of sugars to ethanol, various modifications of the technological process are proposed. Numerous studies have been carried out on the effect of supplementation of VHG substrates with substances having a positive effect on yeast fermentation activity, and the influence of process parameters, i.e. temperature, substrate concentration and yeast dose, on fermentation yield. The effects of the use of various industrial by-products as supplements to VHG substrates providing a source of nutrients were also analyzed. The studies confirmed a significant influence of the concentration and availability of substrates and temperature on the fermentation efficiency. The highest ethanol yield from distillery fermentation is obtained at a temperature above 30 °C, and with appropriately selected initial concentration of fermenting sugars depending on the type of raw material used [1,7–9]. The influence of many supplements (an additional source of nitrogen, metal salts, and vitamins), both for model media and for media prepared from plant raw materials, was also analyzed. The positive effect of the addition of both amine nitrogen (yeast extract, peptone, single amino acids) and ammonium nitrogen (urea, ammonium sulphate) on the degree of attenuation of VHG substrates was found to be dependent on the concentration of the applied supplement [1,5,6,9–13].

Due to the high costs of supplements (such as yeast extract, peptone, metal salts), analyzes of application of industrial waste products were also carried out. These products are used as supplements for VHG media, increasing the efficiency of utilization of the basic raw material and alcoholic fermentation itself. In studies using model media as well

* Corresponding author.
E-mail address: dawidmikulski@ukw.edu.pl (D. Mikulski).

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Table 1
The content of the main components in the raw materials used in ethanol production.

Raw material	Component [%]					References
	Starch	Sucrose	Fat	Ash	Protein	
Potatoes	12 – 21	–	0.1 – 0.3	0.5 – 1.5	1.2 – 3.2	[20]
Wheat	60	–	2.0	1.8	11.7	[20]
Rye	56 – 58	–	1.7	1.9	11.6	[20]
Maize	60 – 62	–	3.7 – 5.0	1.2 – 1.5	2.2 – 8.4	[20,40,41,42]
Sorghum	63	–	2.2	2.8	11.8	[20]
Cassava	58 – 63	–	3.0 – 4.5	1.5 – 3.0	9.0 – 12.0	[20]
Beet Molasses	–	49.7 – 51.0	0.6	8.7 – 12.6	6.0 – 13.0	[20,43]

as substrates from vegetable raw materials, the positive effect of the addition of ingredients present in soy flour, yeast autolysate, spent brewer's yeast and corn steep liquor on the fermentation process efficiency was confirmed [4,11,12,14].

Analyzing the impact of the availability of trophic substances (i.e., fermentable sugars, nitrogen, vitamins, metal ions, etc.) in homogeneous (one-substrate) VHG media, in the context of possible need for supplementation, it is necessary to take into account some limitations resulting from the specific chemical composition of each vegetable raw material used. Studies using various starch raw materials for the preparation of VHG substrates showed significant differences in the efficiency of the fermentation process carried out with the use of rye, wheat, triticale, barley and potatoes [11,15–18]. Differences in the course and efficiency of fermentation carried out with the use of various plant raw materials at similar process parameters result from differences in their composition and availability of extracted nutrients. Traditionally used plant raw materials differ significantly in the content of both basic substances, i.e. carbohydrates, proteins, fat, as well as chemical elements, the concentration of which depends on the variety, vegetation conditions and agrotechnical procedures (Tables 1 and 2). Although each of the raw materials contains substances necessary for the proper course of the alcoholic fermentation process, there are significant differences between the raw materials in the proportions of carbohydrates, proteins, fat, metal ions and vitamins. The availability of these ingredients for yeast is also important. It can be limited, for example, by the presence of complexing compounds in the raw material such as phytic acid [19]. In practice, it is not possible to select the ideal, low-cost plant raw material characterized by a high content of carbohydrates available for the fermentation process and at the same time balanced in terms of other essential nutrients, which used without supplements could guarantee a high efficiency of the fermentation process under VHG conditions. In North America, corn is commonly used for the production of ethanol. Corn is rich in starch and fat, but has a low content of minerals and protein in comparison with other raw materials. In Europe, rye and wheat grain is often used for the production of ethanol. This raw material is rich in minerals and protein, but it is characterized by low content of fat and biologically active substances. Beetroot molasses is a rich source of some minerals and biologically active substances (i.e. biotin, pantothenic acid, meso-inositol) (Tables 1 and 2) [20,21].

The use of particular plant materials for the production of ethanol under VHG conditions, because of the limitations mentioned above, resulting from individual differences in chemical composition, is inherently associated with the need to supplement the media with additional sources of nitrogen, vitamins, fatty acids, growth promoters and stimulators of yeast fermentation activity, which enable achieving a high degree of bioconversion of sugars to ethanol. This is due to the fact that from the technological point of view there is no universal plant material, optimal in terms of chemical composition and proportion of ingredients, which can satisfy all trophic needs related to high yeast fermentation activity under VHG conditions.

An attempt to overcome the above-mentioned limitations resulting from the use of individual raw materials for mashing inspired the present work. The main goal was to assess the possibility of improving the efficiency of fermentation by using a medium that is a complementary optimized composition of various raw materials (maize, rye, wheat and molasses) for use in the fermentation process under VHG conditions. The influence of different proportions of particular raw materials of VHG media on the degree of conversion of sugars to ethanol, fermentation yield and changes in the concentration of volatile by-products was determined.

2. Material and methods

2.1. Raw materials

In our research, we used four raw materials popular in the distillation industry, i.e. corn, rye, wheat, and beet molasses. Raw material samples were examined for macro- and micronutrient content (Table 3). The grain of maize, rye, wheat and beet molasses were characterized by the following parameters: dry matter content [%]: 87.87 ± 0.53, 89.34 ± 0.08, 87.83 ± 0.06, and 85.16 ± 0.26; ethanol yield [L EtOH 100 kg^{-1} of raw material]: 34.75 ± 0.25, 33.00 ± 0.50, 33.50 ± 0.00, 25.75 ± 0.25, respectively. The starch content in maize, rye and wheat grain was [%]: 53.22 ± 0.39, 50.54 ± 0.77, 51.22 ± 0.34, respectively. The sucrose concentration in beet molasses was 49.70 ± 1.21%, and the extract [°Brix] was 81.30 ± 0.40. The acetic acid concentration in molasses (7.10 ± 0.02 g L^{-1}) was determined by HPLC (High Performance Liquid Chromatography) using a refractometric detector [22]. Concentration of the following compounds in molasses was determined using DAD (Diode Array Detector) at 220 nm according to the analytical protocol for Titan C18 columns (Supelco, Pennsylvania, USA): biotin (0.11 ± 0.01 mg kg^{-1}), inositol (5000.1 ± 123.2 mg kg^{-1}), calcium pantothenate (72.2 ± 5.8 mg kg^{-1}) pyridoxine (4.6 ± 0.8 mg kg^{-1}), nicotinic acid (31.4 ± 5.9 mg kg^{-1}) and choline (351.4 ± 29.9 mg kg^{-1}).

2.2. Microorganism

Saccharomyces cerevisiae yeast, strain Ethanol Red (Lesaffre Advanced Fermentations), was used in the form of an active

Table 2
Nutrient concentration in the raw materials used in ethanol production.

Raw material	Concentration of macro- and micronutrients in the grain									References
	N g kg^{-1}	P	K	Mg	Ca	Fe mg kg^{-1}	Mn	Cu	Zn	
Wheat	20	2.0 – 4.3	2.2 – 3.5	0.6 – 2.0	0.4 – 0.7	37 – 53.6	30.0 – 34.4	4.0 – 6.5	34.5 – 56.5	[44,45,46,47]
Rye	14.5	2.9	4.6	1.3 – 2.5	0.5	37.7	27.7	5.0	34.7	[47,48]
Maize	11	2.9	3.9	1.1	0.07	17.3 – 20.0	3.6	1.2	20.3 – 25.0	[44,49]
Beet Molasses	14	0.2 – 0.7	20 - 70	2.4	10 - 50	13.9 – 157.7	2.53 – 21.7	0.5 – 11.0	7.0	[9,20,50,51]