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FERMENTABLE CARBOHYDRATES DETERMINATION IN DIFFERENT WORTS BY HPLC-RI

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ABSTRACT:
The aim of this research was to examine the content of fermentable carbohydrates (glucose, fructose, maltose, maltotriose) in worts produced from different reaction mixtures of cereals. Barley wort and reaction mixtures of the barley wort and other cereals (splitted corn grains, rice and raw barley) are exposed to the extraction procedure (decoction) in acid pH, with addition of the commercial enzyms. Samples of the reaction mixtures of wort and supplement cereals in the amount till 30% were prepared. Fermentation degree and iod value were determined in the samples by standard methods used in beer industry. Fermentable carbohydrates were determined by HPLC-RI methods. The results show very high degree of enzyme hydrolytic dissolution of starch, what is obvious from iod values. The highest concentration of the total fermentable carbohydrates is present in the wort with splitted corn grains and rice. Comparison of fermentation degree and concentration of the total fermentable carbohydrates can show that the concentrations of total fermentable carbohydrates correlate to fermentation degree in the same worts, what says that fermentation degree is a good indicator for total fermentable carbohydrates content. Increase of maltose percentage in worts with supplement cereals is in negative correlation with percentage contents of maltotriosis and glucose (content of fructose is low and pretty equalized).
KEYWORDS:
cereal mixtures, wort, fermentable carbohydrates, HPLC-RI

1. INTRODUCTION

In the production process of beer wort the most important is enzymatic hydrolysis of starch. The aim of hydrolysis of starch is creation of fermentable carbohydrates (glucose, fructose, maltose, maltotriose) which yeasts (leavens) can ferment. Except mentioned low molecular carbohydrates, wort contains small amount of fructose (which rises from free cereal sacharose) and also more decomposable products which leavens cannot fermentate (decstrins). Hydroliytic starch decomposition proceeds in acid pH (by aminolitic enzyms effecting) in three phases: starch gelatinization, partial enzymatic hydrolysis of amylosis and amilopectin till decstrin, and complete enzymatic hydrolisis of fermentable carbohydrates. Gelatinous starch does not exist in the form of compact granules, so it is easy accessible to the enzym`s act [1].

In the worts production, usage of other cereals (together with barley worth) demands the modification of the extraction process. All endosperm ingredients in corn, raw barely and rice, have primary shape because they do not undercome pretreatment process. In those endosperm ingredients there is no synthesis of new

enzyms, nor activation of existing. Therefore, all kinds of supplement cereals must be prepared, so that appropriate enzymatic transformation of endosperm ingredients could be achieved [9]. This can be obtained by thermal digestion of grinded cereal water solution, where it comes to the phase transformation of solution in the form of gelatin. Process runs faster with the addition of barely wort, whose enzyms accelerate decomposition of starch and proteins. This can decrease viscosity of gelatinous solution and accomplish better decomposition of endosperm macromoleculs.

Certain problem research clearly shows that enzyms of wort cannot insure decomposition of appropriate ingredients, nor give the satisfying quality extract. Therefore, the addition of commercial ensyms in worts production with supplement cereals is recommended [13].

2. METHODOLOGY

Cereals used in this research are: barley (Nova Gradiska, Croatia), raw barley (Nova Gradiska, Croatia), corn grits (Mill Ljubače, Tuzla), rice (Monferatto, Italia).

Beside mentioned cereals for producing of fermentable extract-wort, it is used: water (drink water- Water supply system Tuzla), hop (Zalec, Ljubljana), commercial ensyms Termamyl 120 L and Ceramix 2X L (New Nordisk, Switzerland).

Barely wort and reaction mixtures of barley wort and other cereals (corn grits, rice, and raw barley) are conducted to extraction. Prepared samples of reaction mixtures are: 80% of malt and 20% of raw barley (Wort I); 70% of malt and 30% of corn grits (Wort II); 70% of malt, 15% of raw barley and 15% of corn grits (wort III); 70% of malt, 20% of corn grits and 10% of rice (Wort IV) and 100% of barley malt (Wort V). Mashing process of barley wort reaction mixture with supplement cereals (samples I, II, II, IV) is led by the decoction process (52-63-74-100-74°C), by the addition of commercial enzyms (0,05% v/w).

Mashing of barley wort is conducted with decoction without the addition of enzyms. In further procedure extracts are treated with hop (1,2g/L Extract) in STP.

Fermentation degree and iod value determinate by standard methods used in Brewing[11].

The concentrations of glucose (RT – 16 min), fructose (RT – 17 min), maltose (RT – 13 min) and maltotriose (RT - 12 min) in in fermented worts quantified using external standards (Supleco, Belafonte, PA) by HPLC (HP 1050) equipped with a RI detector (HP 1047A)[12,6,3].

The total fermentable carbohydrate content was determined by adding the glucose, fructose, maltose and maltotriose content of each sample. Prior to HPLC analysis, samples were purified using ionic exchange columns (AMINEX HPX-87H), at a flow rate of 0,5 mL/min.

For analyzed extracts are scanned two homatograms. Therefore, results show middle value of two concentrations calculated on 12% of extract. For analyzed extracts are scanned two hromatograms. Therefore, results show middle value of two concentrations calculated on 12% of extract.

3. FINAL RESULTS

The results of iod values measuring in tested worts (Table I), show high degree of enzymatic hydrolitic decomposition of starch. It is probably the effect of commercial enzyms, which are used during the extraction.

Table 1. Jod values in the Worts

Jod value (ΔE_{578})				
Wort I	Wort II	Wort III	Wort IV	Wort V
0,29	0,22	0,20	0,28	0,24

The result of research show that the highest concentration of fermentable carbohydrates in Wort IV, and then follow Worts II, V, III, I. These values are understandable due to composition of cereal worts which are submited to extraction [10].

Table 2. Concentrations of fermentable carbohydrates in the Worts

Worts	Glucose (g/L)	Fructose (g/L)	Maltose (g/L)	Maltotriose (g/L)	Total fermentable carbohydrates (g/L)
Wort I	11,4	11,9	55,0	16,8	85,1
Wort II	13,0	1,9	60,0	18,6	93,5
Wort III	9,3	1,8	58,9	16,1	86,1
Wort IV	14,9	2,0	61,1	20,7	98,7
Wort V	11,5	2,5	56,2	17,2	87,4

The rice contains the highest level of starch, so high content of fermentable carbohydrates in prepared extract with rice portion is expected. However, the problem of enzymatic hydrolysis of rice starch lies in its temperature, which is higher than temperature of pretreated barley.

This is transcended by addition of thermostable a-amylasis, which resulted in considerably high concentration of fermentable carbohydrates in this wort (Wort IV).

A corn is cereal with high amount of starch (higher than pretreated barley and lower than rice).The core of corn starch granules has got higher concentration of amylopectin, while amylose is more concetrated on periphery [4]. Corn starch granules are very sensitive to reaction of amylolitic enzyms and they disintregrate fast [8]. Reaction mixture of corn grits produces extract with high concentration of fermentable carbohydrates (Wort II). Addition of corn grits together with crude barley in wort III, makes the concentration of fermentable carbohydrates bigger than in wort I (in pretreated barely mixture there is only raw barley). In all tested worts, beside mentioned differences, concentration of fermentable carbohydrates has the same values which are considered optional for quality fermentable extract.

It is indisputable that this is the consequence of added commercial enzymes in the extraction process of reactive cereal mixture. Testing the hydrolysis of barely starch with the addition of amylolitic enzymes, Lauro and associates, also Becker and associates have found better hydrolitic decomposition amylopectin than amylose. Reason for that, they point out, is the existance of amylose-lipid complexes, which slow down barley starch gelatinization, and this slows down amylolitic defragmentation activity of amylase [2,5]. Addition of amyloglukosidase during the process of different reaction mixtures affects considerably the increase of concentration of fermentable carbohydrates [14]. Comparing the fermentation degree and concentration of total carbohydrates, it can be noticed that concentration of total fermentable carbohydrates correlate with the fermentation degree in the same worts (Figure 1), which show that fermentation degree is a good indicator of fermentable carbohydrates.

This is of course, significant in everyday laboratory control of brewery, taking into consideration expensive HPLC method which is used in determining fermentable carbohydrates.

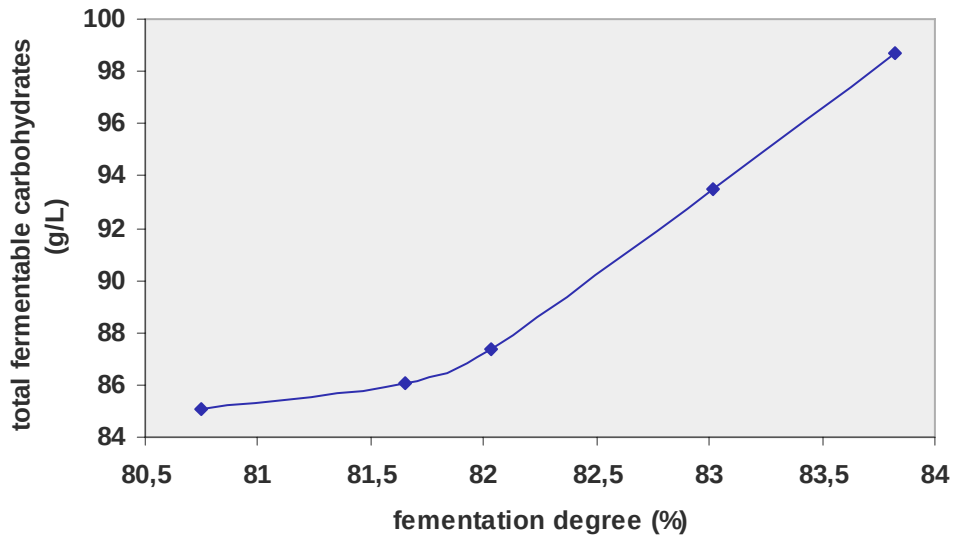

FIGURE 1. Relationship between fermentation degree and total fermentable carbohydrates in the Worts

Mathematical modeling of simulated process enzymatic hydrolysis of maltodekanose, Wojciechowski and associators, have concluded that the beginning stage of hydrolysis directly affects the composition of final product. In all possible combinations content of maltose dominant [7].

Comparing a percentage of glucose, fructose, maltose and maltotriose in tested worts, it is detected higher degree of maltose in relation to the rest of carbonhydrates (Figure 2).

This is the result of β-amylase activity of pretreated barley. Optimal percentage of fermentable carbohydrates in beer wort is 9-12% of glucose, 1-2% of fructose, 50-75% of maltose, and 10-15% of maltotriose. Higher degree of maltose, in worts I and III, is the result of lower gelatinization temperatures of raw barley and corn grits compared to rice. This is optimal for performance of raw barley β-amylase. The smallest precentage of maltose is in wort IV, is becuse of high gelatinization temperature of rice starch. Here β-amylase is inactivated.

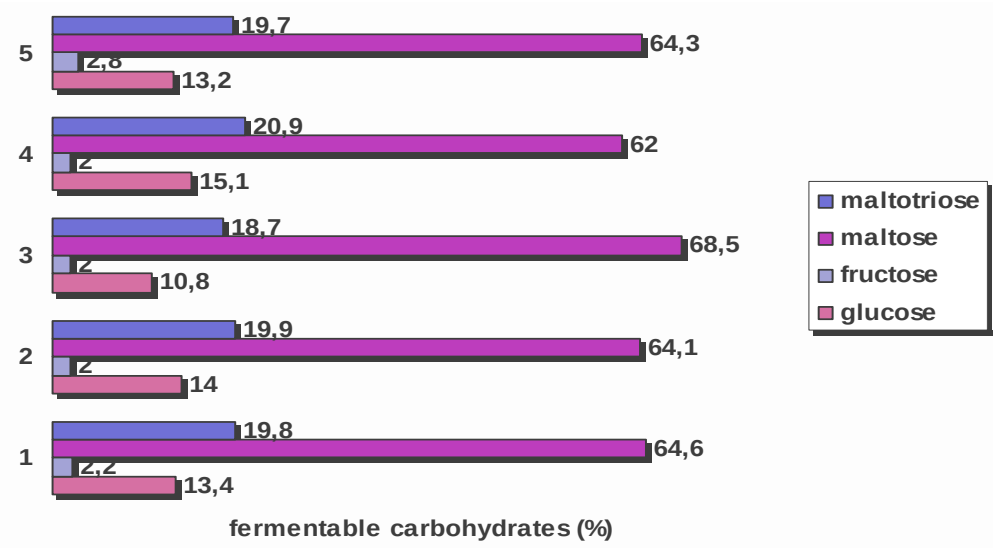

FIGURE 2. Percentage of fermentable carbohydrates in the Worts

Higher precentage of maltotriose is detected in all worts comparing to marginal values for fermentable beer extract. These values are the result of enzym`s addition (Termamyl 120 L), that affects the deeper decomposition of starch granules. This is noticeable in worts II and IV. The same extracts have the bigger percentage of glucose, which can affect the extension of fermentation process (Figure 2).

In this paper has been determined, that the increase of maltose in fermentable extracts with supplement cereals, is in negative correlation with the maltotriose and maltose (fructose substance is small and considerably homogenized) (Figure 3).

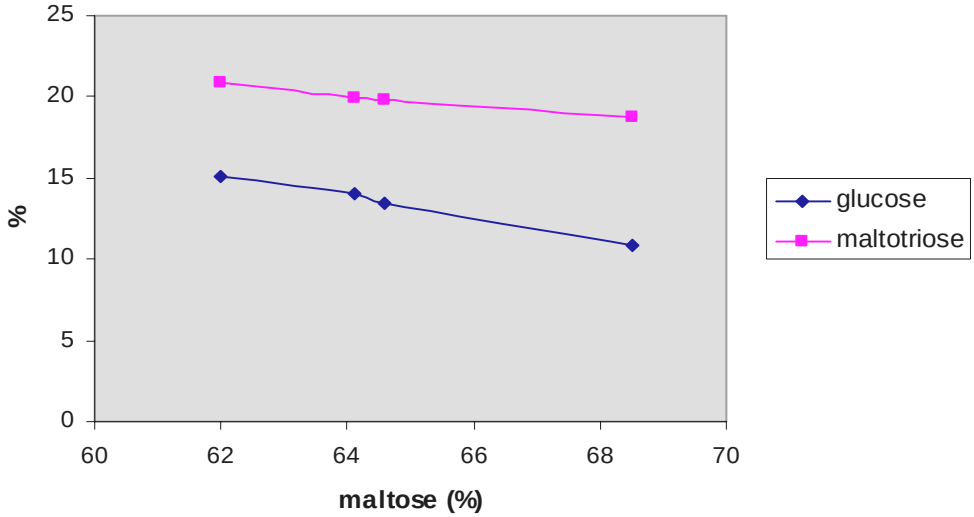

FIGURE 3. Relationship between concentrations of maltose, glucose and maltotriose in the Worts

4. THE CONCLUSIONS

Considerable effect on the cereal starch extraction has the temperature regime of conducting the extraction and pH extract values. An extraction and hydrolitic decomposition of starch in reaction mixtures of cereals are conducted in pH values and temperature conditions, which are optimal for enzymatic performance of amylase that has considerably affected hydrolytic starch decomposition degree. The structure of cereal reaction mixture has direct affect on the concentration of total fermentable carbohydrates. All reaction mixtures produce worts with the highest content of maltose, and then follow maltotriose, glucose, and fructose. The worts of all reaction mixtures have got satisfying concentrations of fermentable carbohydrates. Worts made from reaction mixtures with corn grits portion and rice has higher concentration of glucose than the control sample wort. This can have negative effect on the fermentation process.

The results of conducted research give contribution to extraction testing and enzymatic hydrolysis of starch from biological material. Conducted research shows advantages and disadvantages of substituting some parts of barely starch by supplement cereals in the beer wort production process.

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