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Impact of saccharification method on barley mash fermentation parameters and quality of fermented mash*

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Barley is a comparatively difficult cereal to use as raw material for production of alcohol but it is quite beneficial in commercial terms due to its cost. Barley contains viscosity increasing nonstarch polysaccharides in high concentrations and processing of normal gravity mashes is complicated. Fermentation performance by yeast significantly depends on carbohydrate composition of fermented medium. In this study a laboratory experiment was conducted to ascertain the impact a method of mash saccharification has on fermentation kinetics and quality of fermented mash. Three methods of saccharification have been tested: separate hydrolysis and saccharification, simultaneous saccharification and fermentation (SSF) with one-time dosage of glucoamylase at start of fermentation and SSF with split up dosage of glucoamylase (50% of total amount added at start and the rest — after 12 hours). The kinetics of fermentation was assessed by carbon dioxide release. Glucose and maltose concentrations in mash, medium osmolality during fermentation and congeners concentration at its end were also analyzed to provide a deeper look into influence of saccharification on carbohydrates profile and yeast stress. Maximum fermentation rate was detected in samples with split up glucoamylase dosage in conditions of SSF. Glucose concentration in mash and its osmolality were lowest in these samples during fermentation compared with other methods of saccharification. These samples also showed maximum ethanol yield and minimum total congeners concentration. It is recommended to use SSF technology and split up dosage of glucoamylase during fermentation for production of alcohol from barley if water to grain ratio is 1:2.5.

Keywords: yeast, fermentation, osmolality, glucose, maltose, ethanol.

Влияние метода осахаривания ячменного сусла на показатели процесса его сбраживания и качество зрелой бражки*

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В рамках исследования поставлен лабораторный эксперимент оценки влияния метода осахаривания сусла на кинетику брожения и качество зрелой бражки. Протестированы три метода осахаривания: раздельные осахаривание и брожение, одновременное осахаривание и брожение (SSF) с единовременной дозировкой глюкоамилазы в начале брожения и технология SSF с распределенным дозирование глюкоамилазы (50% от общего количества в начале брожения и оставшееся количество — через 12 ч). Кинетика брожения оценивалась по выделению диоксида углерода. Для полной оценки влияния осахаривания на углеводный состав и стресс, испытываемый дрожжами, были исследованы параметры концентрации глюкозы и мальтозы в сусле, осмоляльности среды в процессе сбраживания и концентрации примесей спирта в зрелой бражке. Максимальная скорость брожения была зафиксирована в образцах с распределенным дозированием глюкоамилазы в условиях SSF. В этих образцах по сравнению с остальными наблюдались минимальные концентрации глюкозы и осмоляльность. В данных образцах также были зафиксированы максимальный выход спирта и минимальная суммарная концентрация летучих примесей спирта. Для сбраживания ячменного сусла с гидромодулем 1:2,5 рекомендуется использовать технологию SSF и распределенное дозирование глюкоамилазы в ходе брожения.

Ключевые слова: дрожжи, брожение, осмоляльность, глюкоза, мальтоза, спирт.

Introduction

Nowadays cutting costs is an important line of potable ethanol industry development.

Barley can be considered an advantageous raw material for ethanol production today because its cost is lowest compared to other types of cereals. Still ethanol industry demands low quantities of this cereal mainly because it is rather hard to process. Barley contains filmy husks with high portion of non-starch polysaccharides which are responsible for increased mash viscosity hampering intensive mass transfer [1–3]. If the cereal is processed by technology of preparation and fermentation of increased gravity mashes this property of barley causes more difficulties. The technology of processing increased gravity mashes is also problematic because these mashes do not reach the needed fermentation completeness which can be identified by residual carbohydrates [4]. One of the factors affecting the concentration of residual carbohydrates in fermented mash is yeast fermenting activity which is in turn dependent on its physiological state [5, 6].

Yeast activity is highly responsive to carbohydrate composition of growth medium. Ustinnikov and Trofimova [7] have shown with respect to high gravity mash that its fermentation completeness is affected by the ratio of concentrations of total fermentable carbohydrates to all the compounds dissolved in mash, and high concentrations of reducing substances have a negative impact on fermentation completeness. It has been already established that not only this ratio but also type of fermentable carbohydrates present in mash has an influence on completeness of high gravity mash fermentation [8–10].

Not only anaerobic but also aerobic paths of yeast metabolism are essential for fast and complete fermentation of mash carbohydrates. The former is responsible for ethanol production and the latter is needed for accumulation of sufficient amount of yeast biomass providing good fermentation dynamics and completeness.

It is known that depending on the type of saccharide its influence on the cell respiratory metabolism is different. The minimum concentration of carbohydrate which initiates the effect of catabolic repression (Crabtree effect) is different for each type of sugar. This concentration for glucose and is higher than that for maltose [11–13]. Thus glucose possesses higher inhibiting action on aerobic cell metabolism compared to maltose.

Yeast physiological activity is also influenced by osmotic pressure or osmolality of medium which is provided by saccharides and other compounds concentrations. This fact has been established during study of brewer's and baker's yeast growth patterns on media of different gravity [14–21].

Taking into consideration that qualitative composition of mash is formed during its preparation [22, 23], it is important to study the influence a method of barley mash saccharification has on fermentation kinetics and quality of fermented mash.

Materials and methods

Grain

The forage barley harvested in 2011 was used for mash preparation. Quality parameters of barley batch used were as follows: moisture content 14.7%, starch content 54.2%, black dockage 1.0%.

Barley was milled on a laboratory roller-mill. Grinding degree can be characterized as 90% pass through sieve with 1mm diameter holes.

Mash preparation and fermentation

Water to grain ratio used was 2.5. Enzymes preparations used for liquefaction included Distizym BA-TS (thermostable α-amylase, dosage 0.3 units of amylase activity per 1 g starch) and Distizym XL (β-glucanase and xylanase, dosage 0.5 units of xylanase activity per 1 g starch).

Enzymes were added to water which was preheated to 50 °C. Milled barley was added to resulting enzymes solution. Mash was kept in water bath at temperature 50 °C during 30 minutes. Temperature was then increased to 70 °C and kept for 90 minutes, and after that — to 90 °C and kept for 60 minutes and then cooled to 60 °C.

The resulting 22% Brix mash was divided into three samples and saccharified with enzyme preparation Distizym AG containing glucoamylase using three different technologies. The dosage was 7 units of glucoamylase activity per 1 g starch for all samples.

The control sample named sample № 1 was saccharified using technology of separate hydrolysis and fermentation (SHF) which means that glucoamylase was added to the sample at 60 °C and the saccharification pause at this temperature lasted 30 minutes.

The remaining two samples were processed without special saccharification temperature stage during mash preparation using technology of simultaneous saccharification and fermentation (SSF). These samples were quickly cooled to fermentation temperature. Glucoamylase was added to these samples at the same time with yeast inoculation and after that. Calculated quantity of Distizym AG was dosed to the sample № 2 entirely at 0 hours of fermentation. The dosage of glucoamylase for sample № 3 was split into two steps in order to decrease glucose concentration at first hours of fermentation: 50% of total amount was added at the same time with yeast inoculum, the rest was added 12 hours later after yeast cells adaptation to the medium.

Fermentation trials were performed in triplicate in 500ml laboratory flasks with water seal in thermostates at 30 °C during 72 hours.

Yeast culture

Yeast strain of *Saccharomyces cerevisiae* was isolated from Ethanol Red commercial active dry yeast (Fermentis), stored on agar solid nutritive medium and used for further cultivation.

The inoculum was prepared by inoculation of yeast material from Petri dish to liquid nutritive medium and further cultivation by fed-batch method without aeration in two stages. For the first stage of cultivation 50 ml of sterile filtered barley malt wort with 12% Brix, prepared from beer wort concentrate, sugar and water, was used as a liquid nutritive medium. For the second stage of cultivation nutritive medium was a 1:1 sterile mixture of described malt wort and molasses solution with 12% Brix. Both stages were performed in thermostat at 30 °C. First stage lasted 24 hours. The resulting culture was added to 350 ml of second stage nutritive medium in full. After 48 hours thermostatting the supernatant was removed from yeast culture precipitate by decantation. Yeast material was then washed with sterile

water, centrifuged at 7.000 rpm during 15 minutes and separated from supernatant two times. Resulting washed culture precipitate was diluted with water to obtain yeast slurry with cells concentration of $2.0 \cdot 10^9$ cells ml $^{-1}$. Mashes were inoculated with resulting yeast slurry when the latter is ready straightaway. Live cells concentration in mash at start of fermentation was $20 \cdot 10^6$ cells ml $^{-1}$.

Fermented mash analyses

Ethanol yield was calculated using following formula:

$$B = Q \cdot X \cdot 100 / G \cdot \sum$$

B — ethanol yield, ml (100 g starch) $^{-1}$;

Q — volume of fermented mash, ml;

X — ethanol concentration in fermented mash,% (v/v);

G — quantity of raw material used, g;

$\sum$ — concentration of conventional starch (fermentable carbohydrates and carbohydrates which can be split into fermentable by high temperature acid hydrolysis measured by polarimetric method and converted to starch by special tables) in raw material,%.

Ethanol concentration in fermented mash was measured in distillate obtained in the course of distillation with areometric method.

Residual soluble carbohydrates in fermented mash were analyzed with photometric method using anthrone [24].

Medium osmolality was measured with osmometer VAPRO («WESCOR Inc», USA).

The amount of carbon dioxide released during fermentation was measured with weight method.

The concentrations of volatile secondary metabolites in fermented mash were measured using gas chromatography method. Chromatograph type Crystal-200M with column HP-FFAP 0.32mm, 0.5μm was used for it. Nitrogen gas with purity of 99.999% was used as mobile gas. Analyses were performed in triplicate. Compound concentrations in one sample varied within ±15% inaccuracy limits.

Results and discussion

The influence of saccharification technology on yeast fermentation activity was assessed by carbon dioxide release kinetics, residual carbohydrate concentrations in fermented mash and ethanol yield.

Fig. 1 represents that during first 8 hours of fermentation carbon dioxide release is more active in samples № 2 and 3 compared to control sample. After 8 hours it can be seen that the intensity of fermentation becomes higher in sample № 3 with split dosage of glucoamylase. This fact can be explained by lower medium osmolality in this sample compared to other samples (Fig. 2).

Ethanol yield was increased by 1.8% when SSF technology combined with split up dosage of glucoamylase were used compared to SSF sample № 2 (Table 1). If it is compared to SHF control sample, it can be noted that ethanol yield grew by 4.7%. The concentration of residual carbohydrates in sample № 3 was reduced by 44% compared to control sample.

Changes in concentration of glucose and maltose in media (Figs 3, 4) were also monitored in order to analyze the combined influence of enzymes action and fermentation process on carbohydrate composition.

Figs 3, 4 represent that the samples had significantly different profiles of changes in fermentable sugars concentrations. The trend of glucose concentration is visually similar to media osmolality changes profile.

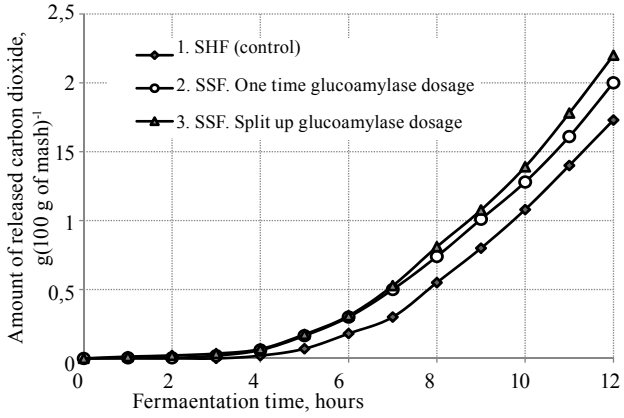

Figure 1. Carbon dioxide release during first 12 hours of fermentation

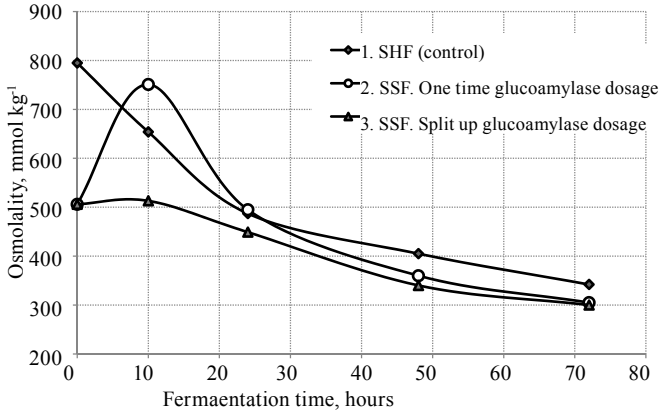

Figure 2. Changes in osmolality of media during fermentation

Table 1

Technological quality parameters of fermented mash

Sample number	Saccharification technology description	Residual carbohydrates, g (100 g starch) $^{-1}$	Ethanol yield, ml (100 g starch) $^{-1}$
1	SHF (control)	1.67	59.8
2	SSF. One time glucoamylase dosage	1.12	61.5
3	SSF. Split up glucoamylase dosage	0.93	62.6

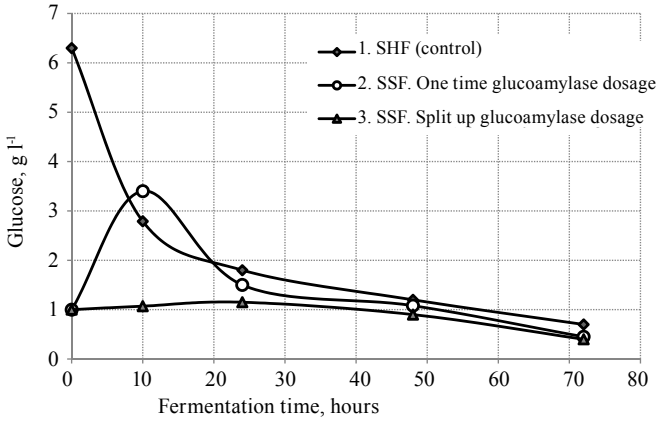

Figure 3. Changes in concentration of glucose in media during fermentation

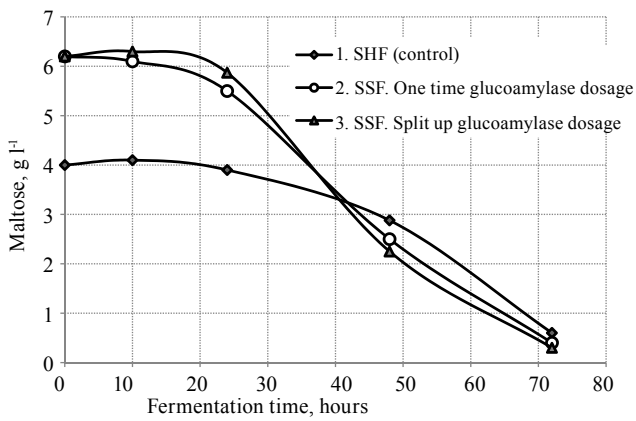

Figure 4. Changes in concentration of maltose in media during fermentation

Table 2

Total congeners concentration and individual compounds concentration in fermented mash samples, ppm

Secondary metabolite	1. Control. SHF	2. SSF. One time glucoamylase dosage	3. SSF. Split up glucoamylase dosage
Acetaldehyde	43.7	7.24	9.6
Acetone	3.43	0.94	0.712
Methyacetate	1.87	1.06	1.78
Ethylacetate	1.54	2.08	3.37
2-Butanone	3.36	1.06	0.81
Methanol	13.8	4.4	4.06
2-Propanol	0.970	0.564	0.505
1-Propanol	23.9	16.1	16.2
Isobutanol	147	142	140
1-Butabol	2.01	1.34	1.37
Isoamulol	643	583	578
1-Pentanol	0.470	0.296	0.320
Hexanol	3.94	1.60	1.57
Acetic acid	156	55.5	42.2
Propionic acid	1.25	1.09	0.83
Isobutyric acid	17.5	8.1	7.47
Isovaleric acid	14.8	8.4	8.0
Valeric acid	17.7	9.8	10.4
Total congeners concentration, ppm	1096	845	827

The maximum glucose concentration was detected in control sample at start of fermentation. In this sample glucose concentration gradually decreases to the end of fermentation and is higher in the final analysis compared to other samples.

For the sample № 2 where SSF technology and one-time dosage of glucoamylase were used the maximum concentration was recorded in 10 hours from the beginning of fermentation and was 3.3%. Glucose concentration in sample № 3 with split up dosage of glucoamylase was lowest compared to other samples throughout the fermentation process.

The profile of changes in maltose concentration in mash during fermentation is completely different from that of glucose concentration. Performing analysis of these data it is necessary to consider that maltose concentration in medium depends on rate of processes including maltose formation during dextrins hydrolysis catalyzed by α-amylase enzyme, maltose utilization by yeast and maltose hydrolysis to glucose molecules catalyzed by glucoamylase. The graphs for samples № 2 and 3 (SSF technology) almost coincide. Maltose concentration in control sample is lower compared to other samples during first 24 hours which can

be explained by enzymatic hydrolysis of maltose catalyzed by Distizym AG.

In order to assess the influence of saccharification method on congeners concentration in fermented mash, distillates were analyzed using gas chromatography method (Table 2).

Minimum total volatile impurities concentration was recorded for sample № 3. SSF technology combined with split up dosage of saccharifying enzyme during fermentation lead to 25% decrease in secondary metabolites content compared to control sample where SHF technology was used.

Table 2 shows that samples № 2, 3 contain less organic acids compared to control sample. Organic acids including acetic, propionic, butyric and valeric acids are indicators of microbial contamination of production process. Acetic acid can also be produced by yeast or be a product of ethanol chemical oxidation [25]. When acetate concentration in growth medium reaches 0.4 g l^{-1} this compound starts to affect yeast physiological state negatively which can lead to «stuck» fermentation and increased ethanol loss with residual carbohydrates [26, 27]. Uncontrolled contamination of fermentation can also cause accumulation of other methabolites of microflora that can impart upredictable organoleptic characteristic to the final product and is therefore inadmissible [28].

Conclusions

The results of this trial show that in case of processing barley mashes with 22% dissolved solids (water to grain ratio 1:2.5) the SSF technology combined with split-up glucoamylase dosage enables to increase ethanol yield from unit of starch weight by 4.7%, reduce losses with unfermented carbohydrates by 44% and reduce total congeners concentration in fermented mash by 25% compared to SHF technology.

SSF technology with one time dosage of glucoamylase also provides advantages over SHF process for the listed indicators but the difference is smaller than in case when split-up dosage of glucoamylase is performed.

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