

Research Concerning Trehalose Use for Wort Fermentation

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Abstract

Changes in environment conditions, like temperature, aeration, type and disposal of nutrients and metabolites concentration, could significantly affect yeast performance during wort fermentation. It was observed that trehalose is a very sensitive response compound synthesized by yeast subjected to stress conditions. The liberated trehalose is either hydrolyzed, being a carbon source for brewing yeast, or both synthesized and stored having the role to stabilize and protect cell membrane. In this article the effect of trehalose add in wort before fermentation on aroma compounds was studied.

Keywords: environment, trehalose, wort fermentation

1. Introduction

All It was shown that stressed yeast cells have a higher trehalose and glycogen content. In other conditions these compounds are degraded. Because trehalose and glycogen don't have structural role they supposedly are reserve materials for yeast. [1, 2, 3] Trehalose acting like a membrane stabilizer is well documented in literature. It was suggested that intracellular trehalose has an important role in yeast cells capacity to tolerate adverse environment conditions. The higher trehalose intracellular content was related to higher osmotic pressure tolerance, temperature tolerance and ethanol tolerance. [14] It was demonstrated that yeasts with higher trehalose content have higher fermentation capacity.

Trehalose accumulation in yeasts occurs as a response to temperature shock and other stresses. It is supposed that this response makes the yeast cells more resistant to stress. For maximum efficiency trehalose must be present both at the external and internal sides of the cell membrane. *Saccharomyces cerevisiae* cells have a trehalose transporter. [3] Some researchers found a strong relation between trehalose intracellular content and yeast capacity to

survive in freezing conditions. [8] It was also demonstrated the correlation of trehalose intracellular content in inverse ratio to glucose induced proton efflux. So by measuring glucose induced proton efflux the stressed yeast population could be detected. [18]

It is a contradiction in literature about yeast cells termotolerance conferred by trehalose or by temperature shock protein. Some authors suggest that termotolerance is induced by intracellular trehalose accumulation independent to shock protein accumulation. Genetic studies demonstrated the implication of trehalose and shock protein in termotolerance protection of yeasts. [8]

Ethanol stress, as temperature stress, leads to intracellular trehalose and unsaturated fatty acids synthesis and total lipid content rising in cells. This rising helps for membrane fortification, leading to structural integrity and a better ethanol tolerance. [14]

It also has been observed that trehalose is one of the most efficient glucids for biological membranes stabilization against

osmotic stress. Brewing yeast uses trehalose as osmoprotective for high gravity and very high gravity worts fermentation. As wort gravity is rising, yeast trehalose content is higher. Trehalose synthesis is probably only one of the mechanisms that confer osmotolerance to yeast. [8] Other authors affirm that glycerol is cell osmoprotective. When subjected to stress some yeasts produce intracellular glycogen during fermentation. [11, 12, 15] It was also been observed that yeast viability depends of its content in trehalose. [16, 17]

Trehalose content determination could be a method for controlling yeast vitality, allowing predicting the fermentative performance. In a study involving 62 yeast strains, most of them could grow on trehalose and could uptake it from the medium and use its protective effect. [10] The trehalose degradation is catalyzed by trehalase and both trehalose synthesis and degradation are stimulated at high temperatures. [11]

2. Materials and Method

Tab To study the effect of trehalose use as yeast protective in wort fermentation industrial wort was used, having the following characteristics: original extract 17.06°P, pH 5.28, color 12.3 EBC units, bitterness 60.8 BU, FAN (free amino nitrogen) content 289.8 ppm, polyphenol content 239.5 mg/l.

The trehalose effect was studied for different original extracts of the wort, different trehalose concentrations and different fermentation temperatures. The samples were prepared like it is shown in table 1. The wort was pasteurized before use.

The wort was corrected to 21°P original extract using industrial maize syrup. The syrup used for experiment had the following characteristics: dry matter content 70.6%, 69.2°Brix, fructose content 41.8% and pH 4.1. The syrup was obtained by maize starch hydrolysis.

Table1. Sample preparation for the study of trehalose use in wort fermentations

Sample	Original extract, °P	fermentation temperature, °C	Initial trehaloze concentration, %
1	17	4	0
2	17	4	1
3	17	4	2
4	17	20	0
5	17	20	1
6	17	20	2
7	21	4	0
8	21	4	1
9	21	4	2
10	21	20	0
11	21	20	1
12	21	20	2

The yeast strain used for fermentation process was industrial slurry yeast and had the following characteristics: viability 97.88%, consistency 34% and pH 4.03. The wort was pitched with 1.5×10^7 cells/ml.

The trehalose solution used for wort supplementation had 50% concentration and was sterilized before use.

The laboratory apparatus used were:

- Karl Zeiss Jena Microscope – for cell counting;
- Analytical balance Owlabor type 750.05 – for weighing the samples;
- Shimadzu gas-chromatograph with capillary column Chromopack 7773, length 50 m, liquid phase AT WAX , detectors FID and ECD, mobile phase N₂/H₂ – for the determination of aroma compounds;
- Anton Paar DSA 5000 – for alcohol content and extract determination;
- Toledo pH-meter;
- CECIL CE 4002 UV-VIS spectrophotometer ;

The methods used were:

- Direct counting of microorganisms with Thomas camera;
- Yeast viability using methylene blue method;

- Gas-chromatographic determination of aroma compounds using EBC method;
- Apparent extract determination with Anton Paar;
- Ethanol determination using standardized method SR 13355-3/1999;
- pH determination using pH-meter;
- Free amino nitrogen determination using ninhydrin method;
- Bitterness determination;
- Polyphenol determination.

3. Results and Discussion

The fermentation process was conducted in 500 ml Erlenmeyer flasks with 200 ml wort having the original extract 17.06°P and 21.84°P respectively.

The trehalose solution was then added in the concentrations showed in table 1 and then the yeast was added. The fermentation process was interrupted after 165 hours and the fermentation was conducted at 4°C and 20°C respectively.

In the final the samples were analyzed for aroma compounds content. The results are presented as it follows.

In the figure 1 the fermentation dynamics is presented and it can be observed that for the samples kept at 4°C a lower CO₂ amount is generated. The aroma compounds determination will also show that the fermentation for these samples is not completed

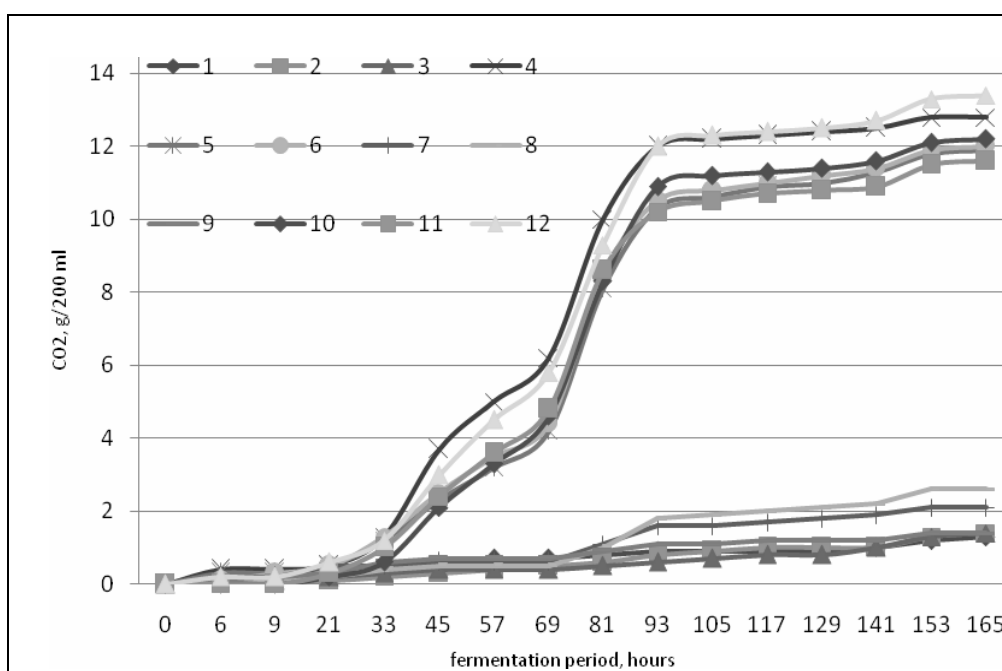


Figure 1. The fermentation dynamics for the samples containing trehalose in liberated CO₂/200 ml wort as a function of time, hours.

The aroma compounds concentration was also determined. Therefore, for the samples fermented at 20°C, the content in diacetyl, 2,3 pentanedione and acetaldehyde is lower than in the samples kept at 4°C showing that the last samples were in a incipient fermentation stage after 165 hours when the diacetyl synthesis dominate, unlike the samples fermented at higher temperature where the diacetyl is reduced to acetoin,

not affected by original extract of the wort. For the samples fermented at 4°C the total vicinal diketones (diacetyl and 2,3 pentanedione) is over the threshold for these compounds.

From figure 2 it can be observed a small raising of the diacetyl content as higher the trehalose concentration is. Also it can be observed lower amounts of diacetyl,

pentanedione and acetaldehyde are formed in 17°P worts comparing to 21°P worts.

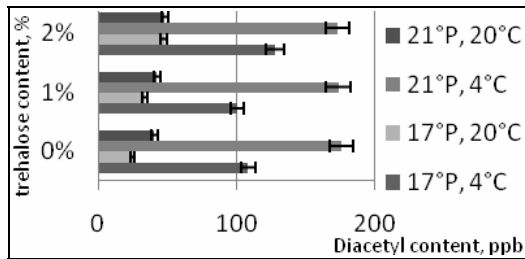


Figure 2. The diacetyl content of the samples after 165 hours of fermentation as a function of trehalose content

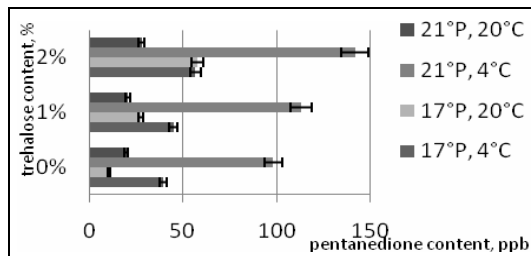


Figure 3. The pentanedione content of the samples after 165 hours of fermentation as a function of trehalose content

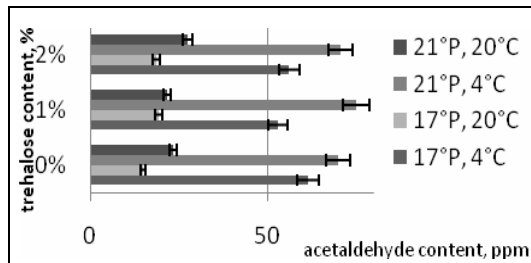


Figure 4. The acetaldehyde content of the samples after 165 hours of fermentation as a function of trehalose content

The diacetyl, pentanedione and acetaldehyde content variations demonstrate the incomplete fermentation process for the samples kept at 4°C. Also, the addition of trehalose seems to have protective role but the differences between the samples with added trehalose and the control samples are relatively small.

As it can be observed in figures 5 and 6, the esters content is higher when the wort was fermented at 20°C, higher fermentation temperatures being an important factor affecting ester synthesis as Derdelinkx et al showed in 2003 and 2004 and Debourg in 2002. [4, 5.6]

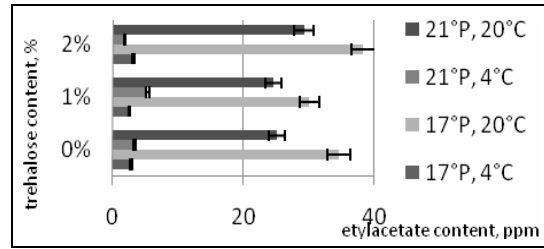


Figure 5. The ethylacetate content of the samples after 165 hours of fermentation as a function of trehalose content

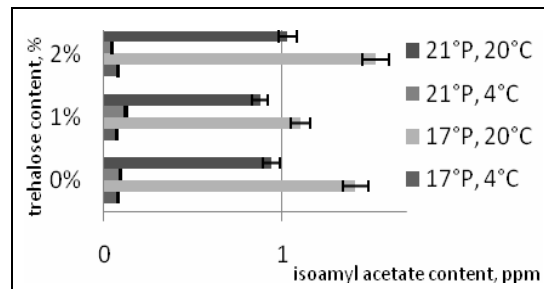


Figure 6. The isoamyl acetate content of the samples after 165 hours of fermentation as a function of trehalose content

It can be observed that when higher fermentation temperature is used the differences between the ester amounts produced are higher. Also, it can be observed that when 1% added trehalose is used a lower amount of esters is produced, being unjustifiable the use of higher trehalose concentration.

The higher alcohols amount is higher when the fermentation temperature is higher. When wort is fermented at 4°C the propanol content is lower for lower original extract of the wort. At 20°C, when 21°P wort is fermented, a lower amount of propanol is obtained, not affected by the trehalose concentration. (Figure 7)

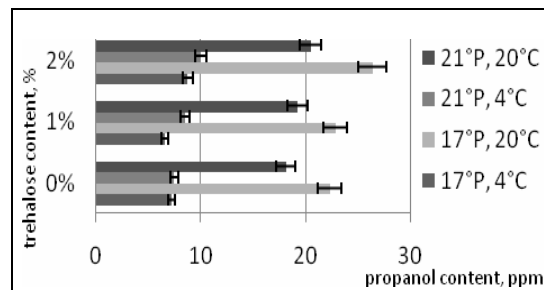


Figure 7. The propanol content of the samples after 165 hours of fermentation as a function of trehalose content

It can be observed that generally, when trehalose is added to the fermentation

medium, the higher alcohols amount is higher. It was demonstrated that the yeast content in trehalose do not affect the extract rising and the ethanol production during fermentation. Higher trehalose content in brewing yeasts however sustains yeast viability during initial phases of fermentation, resulting a better use of glucids and so higher amounts of isoamyl alcohol and isobutanol. [9]

In figures 8 and 9 it can be observed that using higher fermentation temperature higher amounts of isoamyl alcohol and isobutanol are formed during fermentation. Also, in samples with added trehalose higher amount of isoamyl alcohol and isobutanol are formed, these results being obtained by Guldfeldt also. [9]

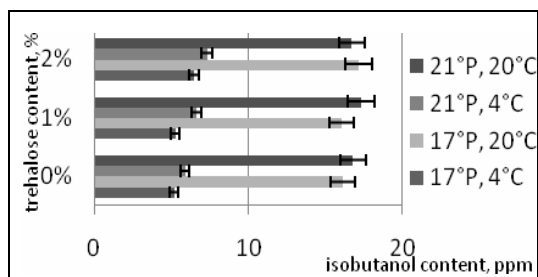


Figure 8. The isobutanol content of the samples after 165 hours of fermentation as a function of trehalose content

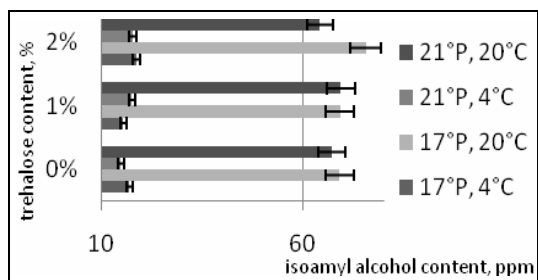


Figure 9. The isoamyl alcohol content of the samples after 165 hours of fermentation as a function of trehalose content

The stimulatory effect of trehalose on higher alcohols synthesis can be explained by rapid uptake of trehalose by yeast cells after inoculation during normal gravity wort fermentation and that they synthesize higher alcohols in order to modulate their redox balance. It is possible that yeast with higher trehalose content will dissimilate trehalose to a greater extent and, taken together with the higher glucide utilization rate, will result in an increased glycolytic

flux. In order to sustain this glycolytic flux the high trehalose containing cells have to provide more NAD^+ and, hence may increase the production of higher alcohols. [9]

4. Conclusion

Trehalose added in the wort before fermentation has a protective effect on yeast cells subjected to different kinds of stress (osmotic, temperature and ethanol stress) especially when high gravity worts are used. Added trehalose affects the aroma compounds synthesis and dynamics. Therefore, when trehalose is added to wort, by protecting yeast cells subjected to osmotic stress due to high gravity brewing, a high fermentative activity is sustained causing the increased production of aroma substances when higher trehalose concentrations are used.

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