MONITORING OF THE APPLE FERMENT PARAMETERS

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Abstract: The aim of this study was to describe an experimental process, of which the main part is the preparation of apple batches and monitoring of the fermentation process. The batch was created from pulped apples to which special yeast cultures were added. Different process parameters of the fermentation were monitored during the experiments, depending on the yeast type and the fermentation temperature conditions. The fermented batch was later used in the distillation process of apple yeast.

KEYWORDS: Fermentation, Yeast, Determination of ethanol

1 Introduction

Fermentation is a process of transforming organic matter, typically saccharides. During the fermentation process, the participation of microorganisms leads to the creation of more energy-poor substances (1). These include substances from the alcohols, carboxylic acids and aldehydes groups. Fermentation can take place in two types of environment, aerobic and anaerobic. Fermentation can also be distinguished on the basis of the resulting product and has wide range of uses. It plays a key role in the food industry (2), in the production of fermented food, the preparation of distillates and wines. It is used in the chemical industry (3), and in the production of biofuels (4) from waste from agricultural and wood industries or from municipal waste and livestock excrement. With the help of yeast additives, it is possible to adjust the resulting nutritional composition and quality of the final product. The choice of yeast and the fermentation conditions are an important element influencing the quality of the final product. In addition to quality, they also influence, for example, the development of sensory properties (5).

2 MATERIALS AND METHODS

As a basis for the experiment was a mixture of apples from own collection. The experiment took place in two series. In the first series, the main observed parameter was the time for which fermentation takes place under the given conditions and used chemicals. The first series of experiments was performed in 4 bioreactors. The bioreactors had the same dimensional parameters, volume 10 l, but different conditions (temperature, yeast used). In the second series, the fermentation was stopped after the ideal fermentation length based on the results from the first series and the parameters in the individual bioreactors were evaluated. Tab.1 and Tab.2 shows the type of chemicals used and conditions at the beginning of the experiments in all bioreactors.

Tab. 1 Distribution of the bioreactors during the 1st series of experiments and conditions at the beginning of the experiment. TP - thermophilic yeast, CP – cryophilic yeast.

	1.series of experiment			
	1.bioreactor	2. bioreactor	3. bioreactor	4. bioreactor
pH regulator	200 ml	200 ml	200 ml	200 ml
pH	3,05	3,10	3,04	3,07
Enzyme	5 ml	5 ml	5 ml	5ml
Nutrient salt	5 g	5 g	5 g	5 g
Yeast	CP – 1,5 g	CP – 1,5g	TP – 1,5 g	TP – 1,5g
Location	Lab.A:24- 25°C	Lab. B: 17 - 18°C	Lab.A:24- 25°C	Lab. B: 17 - 18°C

Tab. 2 Distribution of the bioreactors during the 2nd series of experiments and conditions at the beginning of the experiment. TP – thermophilic yeast, CP – cryophilic yeast.

	2. series of experiment					
	1. bioreactor	2. bioreactor	3. bioreactor	4. bioreactor	5. bioreactor	6. bioreactor
pH regulator	200 ml	200 ml	200 ml	200 ml	-	-
pH	3,06	3,04	3,04	3,02	4,03	3,91
Enzyme	5 ml	5 ml	5 ml	5 ml	-	-
Nutrient salt	5 g	5 g	5 g	5 g	-	-
Yeast	CP – 1,5 g	CP – 1,5g	TP – 1,5 g	TP – 1,5 g	-	-
Location	Lab. A: 24- 25°C	Lab. B: 17 - 18°C	Lab. A: 24- 25°C	Lab. B: 17 - 18°C	Lab. A: 24- 25°C	Lab. B: 17 - 18°C

During the experiment, various devices were used to prepare and evaluate the parameters. A mill was used to grind the raw apples, a METTLER Toledo laboratory pH meter, wine densitometers, and the probes for measuring the change in oxygen value and the redox potential. Measurement of oxygen change and redox potential was performed using a Solaris biotechnology laboratory bioreactor unit. Three types of chemicals were used to treat the fermentation, Erbsloh PH-Senker pH regulator, Distizym FM enzyme, VITAMON COMBI nutrient salt and two types of yeast, Oenoferm Freddo cryophilic yeast (CP) and Spirifer Classic thermophilic yeast (TP). Both yeast species belong to the *Saccharomyces cerevisiae* strain [6]. The pH regulator used is a liquid combination of acids to acidify the yeast from the fruit. It is used to reduce the pH value in yeast to 3.0 - 3.5. It is also used as an infectious protection to suppress unwanted lactic, acetic and buttery fermentation bacteria. The pH regulator used is a combination of citric and lactic acid to promotes pure fermentation and optimizes the development of fruit aromas and bouquet substances. It can be added directly to the yeast, but it is important to mix it thoroughly. The same enzyme was used in all yeasts. It is a pectolytic enzyme for the optimal treatment of mash intended for distillation. It is a standard aid in the production of yeast, developed for high efficiency in the acidic environment of fruit mash. It degrades all pectin substances, which leads to a significant reduction in viscosity and its rapid liquefaction. It also releases valuable substances that form aroma and bouquet and optimizes the course of the fermentation process. It is ideal to add it directly to the crusher. The nutrient salt supplies vitamin B1 and diammonium phosphate to the fermentation. It is used mainly in the production of quality wines and its use achieves optimal fermentation. The first type of yeast used was cryophilic yeast for fermenting in temperature from 8 ° C [8]. They ferment a higher degree of alcohol despite the low temperature. The second type of yeast was thermophilic yeast for standard fermentation

conditions. They are strong fermenting pure yeasts. Prior to milling the raw apples, bioreactors in the form of plastic bioreactors were prepared and sufficiently treated to prevent contamination by fungi and other organisms. The required number of raw apples was cleaned and cut in half. By slicing the apples in half, we ensured better grinding with the mill. After crushing, in the first series of the experiment, the apple pulp was divided into four bioreactors of 10 l each. A pH adjuster, nutrient salt, enzyme and yeast were then added to each bioreactor. In the second series, the apple pulp was divided into six bioreactors of 10 l each. Chemicals were added only to four bioreactors. The other two bioreactors were left to natural fermentation. Bioreactors were in two laboratories. The temperature in Laboratory A was approximately 24-25 °C and in Laboratory B the temperature was 17-18 °C. The difference in the individual batches was ensured by different types of yeast. The yeast differed based on the ideal temperature at which they ensure the highest yield during fermentation. During the experiment, several quantities were measured, and their course was monitored throughout the fermentation period. Based on this, the courses of individual parameters and the effect on fermentation under the given conditions were evaluated. The measured values were temperature, density and redox potential. The values of the quantities were recorded during the selected days. After experiments graphs were constructed with curves of the course of individual quantities during the days when fermentation took place. Based on the measured density values in individual bioreactors, it was possible to determine the sugar content of apple mash at the beginning and the end of fermentation. The sugar content of the batch was determined using a conversion calculator to determine the sugar content of the must for the values of the standard must meter [7]. The alcohol content of the apple mash is then determined by the equation:

$$c = c_0 - c_1^{\circ NM} \quad (1)$$

where c represents the difference between the sugar content of the apple mash at the beginning of the fermentation c_0 and the sugar content of the apple mash at the end of the fermentation c_1 , provided that 1 °NM (normalized must meter) accounts for 0.58% by volume of the alcohol formed. Based on this calculation, the ethanol concentration in the individual bioreactors after fermentation was determined.

3 RESULTS AND DISCUSSION

The trend of the parameters observed during fermentation from the first series of experiments, in which apple pulp was fermented in four bioreactors of 10 l each, are shown in Fig.1-3. The Fig.1 shows trend of temperature in the batch. We can see step change in temperature in the first days. This may be due to a change in ambient temperature. The most important measurement for us was the change in density. Based on which we can assume, the degree of fermentation, as the value of the batch density will gradually approach one. The first series of experiments was followed for 11 days. When measured at the end of the experiment, it was found that the yeast fermented almost all the sugar and acetic fermentation began. Acetic fermentation was evaluated based on observations. The redox potential was measured only as a supplementary data, which expressed the degree of oxidation-reduction events. Over time, the redox potential should decrease as can be seen in Fig.3. After evaluating the data from the first experiment, it was possible to observe the ideal time for fermentation of apple yeast. The time required for fermentation was set at approx. 8 days like in the work of Zuo et al. [7]

Fig. 1 Temperature course in the 1st series.

Fig. 2 Density course in the 1st series.

Fig. 3 The course of the redox potential in the 1st series.

The results of the second series of experiments, which you can see in Fig. 4-6, where similar trends can be observed as in the first series of the experiment. It is also possible to say from the density graph that we achieved approximately the same degree of fermentation in both cases, with the use of yeast and also in natural fermentation. The density of the batch was determined from a sample that did not contain residues of larger solid particles, as only the filtered part is dosed into the boiler during the work. the redox potential also had a grading tendency in this case as in the first series of experiments. The change in ambient temperature and thus the batch had less fluctuating values during the second experiment.

Fig. 4 Temperature course in the 2nd series.

Fig. 5 Density course in the 2nd series.

Fig. 6 The course of the redox potential in the 2nd series.

After stopping the fermentation process, the resulting ethanol values in the batch were determined. Based on which it is possible to evaluate the most effective combination to achieve the highest ethanol content from the fermentation. The determined values of ethanol in individual batches can be seen in Tab.3. The amount of ethanol was determined only after the second series of experiments.

Tab.3 Amount of ethanol in individual batches

Calculation of the amount of ethanol						
i	1. Bioreactor	2. Bioreactor	3. Bioreactor	4. Bioreactor	5. Bioreactor	6. Bioreactor
C0 [°NM]	10,2	12,2	8,7	11,0	10,2	11,0
C [°NM]	1,0	2,2	0,8	1,0	0,1	5,2
ethanol [%]	5,3	5,8	4,5	5,8	5,8	3,3
Average amount of the ethanol [%] in bioreactors				5,1		

As part of further research, tests would be performed to evaluate the quality of distillates based on their taste.

CONCLUSION

Findings of the first experiment series was determination of the apple pull fermentation time at different conditions and used chemicals. This finding was confirmed by several repetitions in an effort to ensure similar conditions. After the first series the method of reading the change in the amount of dissolved oxygen was changed because the first experiment method was based on average values. On the other hand, the change in the amount of dissolved oxygen was read for a specified time. The fermentation is exothermic process so there was assumption that a temperature will rise. This assumption was confirmed by measured results. Also, there was assumption that the apple pull density will be around 1 kg/m³. This is caused by the gradual transformation of sugar to ethanol. It is also due to a fact that the mixture will be filtered after fermentation to remove solids. The resulting values of the ethanol content in the batch are shown in Tab.3. The average value of the ethanol concentration in the batch was used as the input concentration in further experiments. This value of the ethanol concentration was determined on the basis of the measurement of the fermentation density and the subsequent calculation. The calculated value agrees well with the data observed from practise, which were provided by the operator of the experimental station. The average value apple mash distilled on the plant reaches the level of 5% by volume of ethanol. To determine the most suitable working procedure, in this case we will use the

ethanol yield from the individual batches. Based on this, the highest yield was in bioreactors 2, 4 and 5. From an economic point of view, it would be a batch in bioreactor No. 5, as without the addition of chemicals, it achieved an ethanol yield comparable to the inoculated batches.

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REFERENCES

- [1] Kirchmayr, M. R., Segura-García, L. E., Lappe-Oliveras, P., Moreno-Terrazas R., de la Rosa, M., Gschaedler Mathis, A. "Impact of environmental conditions and process modifications on microbial diversity, fermentation efficiency and chemical profile during the fermentation of Mezcal in Oaxaca", *LWT - Food Sci Technol.*, **2017**.
- [2] Matejčeková, Z., Liptáková, D., Valík, Ľ. "Functional probiotic products based on fermented buckwheat with *Lactobacillus rhamnosus*", *LWT - Food Sci Technol.*, **2017**.
- [3] Sun, X., Atiyeh, H. K., Huhnke, R. L., Tanner R. S. "Syngas fermentation process development for production of biofuels and chemicals: A review", *Bioresource Technology Reports*, **2019**.
- [4] Kumar, D., Juneja, A., Singh V. "Fermentation technology to improve productivity in dry grind corn process for bioethanol production", *Fuel Process Technol.*, **2018**.
- [5] Magic MM. Incat.pdf [Internet]. **2017**. Available from: <http://www.previnarov.sk/>
- [6] Zuo, W., Zhang, T., Xu, H., Wang, C., Lu, M., Chen, X. "Effect of fermentation time on nutritional components of red-fleshed apple cider", *Food Bioprod Process*, **2019**.
- [7] Gužela, Š., Juriga, M., Fekete, R. "Influence of particle size of particulate matter on the angle of internal friction", *Strojnícky časopis – Journal of Mechanical Engineering* 60 (2), pp. 105 – 120, **2009**. ISSN 0039-2472.
- [8] Macho, O., Čierny, M., Gabrišová, Ľ., Juriga, M., Ružinský, R., Peciar, P. "Dynamic Image Analysis to Determine Granule Size and Shape, for Selected High Shear Granulation Process Parameters." *Strojnícky časopis – Journal of Mechanical Engineering* 69 (4), pp. 57 – 64, **2019**. DOI: 10.2478/scjme-2019-0043