THE EFFECT OF HOP α-ACIDS ON THE ALCOHOLIC FERMENTATION PROCESS AND THE ETHANOL YIELD

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Abstract: The alcoholic fermentation is exposed to a high risk of microbial infections, which have a significant impact on the efficiency of the process and the quality of the distillates. This article presents the effect of lactic acid bacteria on an alcohol fermentation by yeast and a method of reducing this undesirable microflora with the use of a preparation of hop α-acids. The results indicate that the application of hop α-acids preparation allows reducing the development of microbial infection, which are mainly lactic acid bacteria. Besides, it allows an ethanol yield to be improved. The solution is as a pro-ecological and agreeing with EU politics.
Keywords: hop α-acids, fermentation, native starch, lactic acid, rye

Introduction

Bacterial contaminants in alcoholic fermentation lead to the formation of undesirable by-products and cause losses in the efficiency of alcohol. Lactic acid bacteria are the most troublesome microorganisms occurring during the production process of agricultural distillate, because they develop quickly in the fermentation environment, with the presence of carbon dioxide, especially in the phase of yeast propagation, at 30-40 °C, at low pH values. The causes of pollution may depend on the purity and quality of raw materials, yeast, fermentation tanks, transmission lines, heat exchangers, etc. (Reed & Nagodawithana, 1991). In factories producing malt whiskey, in which mash is not boiled to preserve the activity of natural malt enzymes, bacterial contamination may deteriorate the quality of distilled spirit and reduce the final yield of this high-quality fermentation product (Walker & Hill, 2016). The strains of lactic acid bacteria isolated from samples from the distillery environment were characterized by high activity during alcoholic fermentation, probably as a result of their adaptation (Bischoff, Skinner-Nemec, & Leathers, 2007; Schell et al., 2007; Skinner & Leathers, 2004). In industrial practice, determining the number of bacteria in many distilleries is often limited to the detection of lactic acid, because aerobic and facultative anaerobic bacteria with low pH tolerance are not considered a serious threat to product quality and production efficiency. The number of bacteria can be significantly reduced by cleaning and disinfecting the equipment, keeping mash at temperatures above 70 °C, using pressure-thermal methods or chemically sterilizing the substrates and adding antibiotics such as penicillin (Ralph, 1981; Rückle & Senn, 2006) or virginiamycin (Arshad, Zia, Asghar, & Bhatti, 2011; Islam, Toledo, & Hamdy, 1999). However, this is doubtful from an economic point of view, but it is even more important to raise awareness and thus also fears of the growing spread of bacterial resistance due to the huge abuse of these compounds. Despite the above precautions, bacterial contamination still occurs in many ethanol plants. Large counts of bacterial cells cause a decrease in the growth and metabolism of yeasts, caused by competition for available nutrients, but also the excretion of toxic metabolites, such as lactic acid in the case of bacteria (Oliva-Neto & Yokoya, 1994). Therefore, it is necessary to take actions to limit undesirable microflora in ethanol fermentation.

All conventional antibacterial agents in the production of ethanol show some deficiencies in the antimicrobial activity (depending on the type of bacteria, selectivity, and yeast status, chemical stability in fermentation conditions (Essia Ngang, Letourneau, & Villa, 1989) or environmental safety concerning animal and human health. that the European Union has banned the use of antibiotics as bactericidal compounds, including in the distillery industry, because the remaining decoction is used as animal feed.

Despite many research efforts aimed at reducing undesirable bacterial flora, this is still a serious problem that poses a threat to the dynamic development of commercial alcohol production. Traditional methods for keeping bacterial contaminants at an acceptable level include introducing a very low pH, for example between 2 and 3 (Gibson, Lawrence, Leclaire, Powell, & Smart, 2007) with sulfuric acid (H2SO4). Proecological techniques are based on the age-old knowledge about the hop, which can provide not only a beneficial taste of various beverages, but also protection against the development of bacterial microflora (Simpson & Smith, 1992; Suzuki, Iijima, Sakamoto, Sami, & Yamashita, 2006).

The antibacterial properties of hop (*Humulus lupulus*) have been known and used in the brewing industry for ages (Verzele & De Keukeleire, 1991).

The topic of this work is to present the effect of hop α-acids in inhibiting the development of unfavorable bacteria during the alcoholic fermentation and yield of the process.

Materials and Methods

For preparing the mashes, rye of the variety Dańkowskie Amber (Danko Hodowla Roślin sp. O.o., Choryń) was used. The physical and chemical analysis of the raw material, including determination of dry mass, protein, reducing sugars and starch content was carried out following the methods recommended in the agri-food industry (AOAC, 1995).

Starch hydrolysis was carried out using amylolytic preparations such as: GC 626 liquefying enzyme preparation, containing the acid α-amylase (EC 3.2.1.1), derived from *Trichoderma reesei*, at a dose of 0.3 mL per 1 kg of raw material and the second saccharification preparation Stargen 002, which contains a blend of *Aspergillus kawachi* α-amylase (EC 3.2.1.1) expressed in *Trichoderma reesei* and glucoamylase (EC 3.2.1.3 from *Trichoderma reesei*) in an amount of 1.2 mL per kilogram of raw material. All the enzyme preparations were purchased from DuPont TM Genencor® Science (USA).

A commercial preparation of dry distillery *Saccharomyces cerevisiae* yeast, Ethanol Red (Fermentis, Division of S.I. Lesaffre, France), was used at 0.3 g L^{-1} mash. The yeast was hydrated and disinfected (15 min, at room temperature) using water and sulfuric acid (25% w w-1) solution (pH of yeast slurry was set at 2.0). Along with the yeast medium, a mineral nutrient for yeast - an aqueous solution of $(NH_4)_2HPO_4$ at a dose of 0.2 g L^{-1} mash was added. A preparation IsoStab® (BetaTech, Germany) of hop α-acids in an amount of 140 ppm was added.

The mashes were prepared with pressureless liberation of starch method (PLS), using a previously ground raw material, in a mill equipped with corundum and ceramic grinders (crumbling below 1.5 mm). The process was carried out in a mixer equipped with a stirrer disposed of in the water heating mantle.

The hydrolysis of native starch consisted of mixing the ground grain with water (in a ratio of 1: 2.8), the pH was adjusted to 4.0 with use of a solution of sulfuric acid 25% w w^{-1}, then the mixture was heated to a temperature of 35 ± 2 °C, and next the GC 626 enzyme preparation was added, these conditions were maintained for 30 minutes in order to pre-hydrolyze a starch(so-called ‘activation'). Then, stirring constantly, the mash was cooled to the fermentation temperature (35 °C) and the pH was controlled and possibly re-adjusted to 4.0, after which STARGEN 002 was added. For the samples of mashes of the second variant, the hop α-acid preparation was additionally added. Then all the samples were treated with yeast and nitrogen medium. The process was conducted at 35 °C for 3 days.

During fermentation, samples of the mash were collected (every 24 h) to determine the content of sugars and ethanol. Once fermentation was complete, the ethanol was distilled from the mash using a laboratory kit consisting of a Liebig cooler, a flask, and a thermometer. The distillates obtained, containing 20–30% (v v^{-1}) ethanol, were strengthened to ethanol contents of approximately 43% (v v^{-1}) in a glass distillation apparatus with a special dephlegmator/condenser, according to the method described by Golodetz (Hulda, Njintang, & Cmf, 2017).

The raw materials were analyzed to determine its content of moisture, total nitrogen (AOAC, 1995), reducing sugars (Pomeranz & Meloan, 1995) and starch (BS EN ISO 10520:1998, 1998) using recommended methods in the agricultural and food industries.

Before and after fermentation, the contents of total sugars and reducing sugars in the distillery mashes were determined according to the recommended methods for the distilling industry (AOAC, 1995) expressed in g of glucose per L of mash. Dextrin content (expressed in g L^{-1} of mash) was calculated as the difference between the amounts of total sugars and reducing sugars, taking into account the conversion coefficient (0.9) into dextrins.

Analysis of the sugar profile was performed on high-performance liquid chromatography (HPLC). An Agilent 1260 Infinity apparatus (Agilent Technologies, USA) was used, equipped with a refractive index detector (RID) (temperature set at 55 °C). A Hi-Plex H column (7.7 × 300 mm, 8 µm) (Agilent Technologies, USA) was used to separate the compounds. The column temperature was maintained at 60 °C. As a mobile phase, 5 mM of H2SO4 was used at a flow rate of 0.7 mL min-1. Before analysis, mash samples were deproteinized and filtered through a 0.45 µm PES (polyethersulphone) membrane, then injected at a volume of 20 µL (Hulda et al., 2017)

(Strąk-Graczyk & Balcerek, 2019)

Based on the obtained results, the fermentation factors were calculated, i.e. the intake of sugars and fermentation yield (in relation to total sugars determined in the sweet mash).

Results and Discussion

The raw material used in the research was characterized by a dry matter (d.m.) content at the level of 87.1±1.7%. With reference to literature data (Pietruszka & Szopa, 2014), it indicates that the tested raw material was characterized by low water content (Tab.1). In the case of distilleries working in the all-season system, the moisture of the raw material is a significant parameter, which affects possible storage of cereal grain. The processed raw material contained a protein at the level of 11.9±0.2%, which was higher than in rye grains described in the literature. On average, the total protein content in cereals is between 10% and 12% (w w^{-1}) (HGCA, 2018) but the kinds of protein in each variety of cereals have not accurately been described (Villegas-Torres, Ward, & Lye, 2015). Only about 10% – 15% of the total protein is dissolved during mashing. The unhydrolyzed residue remains along with leftovers from the raw material in the medium (Bringhurst & Brosnan, 2014). The content and types of proteins in grains affect the ethanol yield, which is related to the degree to which the starch granules are embedded in the protein matrix. The strength of protein adhesion and the biomechanical properties of the layers cereal grains play an important role in the processing of the raw material, and subsequent hydrolysis of the granules (Agu et al., 2012).The rye used for research was characterized by starch content at the level of 68.5±1.4%, which is similar to the literature data (Pietruszka & Szopa, 2014) and indicates the usefulness of grain for distillation purposes.

Table 1: The physicochemical composition of rye grains.

Raw material	Dry matter [%]	Protein [% d.m.]	Reducing sugars [g glucose/100 g raw material]	Starch [g/100 g raw material]
Rye Amber cultivar	87.1±1.7^{b}	11.9±0.2^{b}	1.8±0.4^{a}	68.5±1.4^{a}
Literature data (Pietruszka & Szopa, 2014)	80.5±0.02^{a}	9.4±0.05^{a}	4.4±0.01^{b}	67.8 ± 0.3^{a}

Different superscript letters in columns indicate significant differences (P <0.05) between mean values.

Based on the results of the analysis of reducing and total sugars concentrations in the medium after pre-hydrolysis, the efficiency of starch saccharification ('activation') was calculated and expressed in % of its total content. This allowed the evaluation of enzymes performance depending on the variant of prepared samples. Figure 1 shows the results of pre-hydrolysis of rye starch.

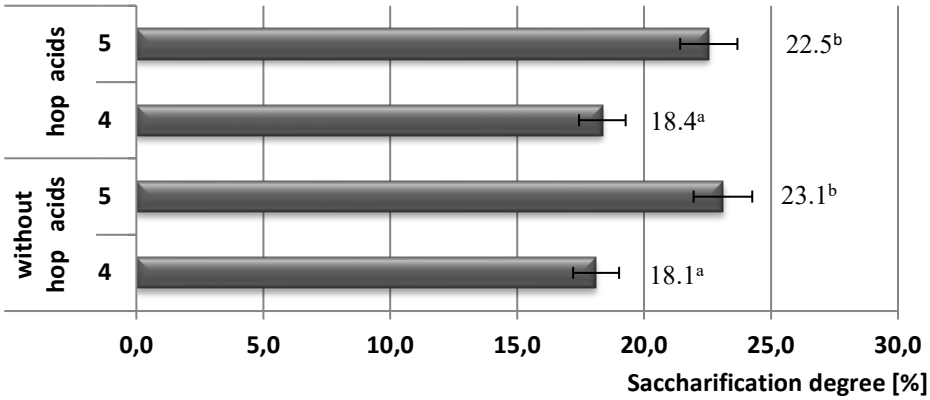

Figure 1 Efficiency of initial rye starch saccharification for two initial pH of medium (pH 4.0 or pH 5.0), and without or with the addition of hop α-acids preparation. Different superscript lowercase letters indicate significant differences (P <0.05) between mean values.

Because hop α-acids preparation was added just before the task of yeast testing, we cannot determine their effect on the starch 'activation' step. However, by this indicator, it can be concluded that the acid α- amylase present in the enzyme preparation GC 626 used for the initial hydrolysis showed higher activity in mashes with initial pH 5.0. Chemical analysis of sweet (Tab. 2) and fermented (Tab. 3) mashes included pH measurement and evaluation of sugars (glucose, maltose, maltose), and dextrins concentrations. Moreover, samples were taken every 24 h to determine the lactic and acetic acid concentrations (as indicators of microbial infection) and ethanol content.

Table 2: Chemical composition of sweet mashes.

Mashes before fermentation	pH	Concentration of sugars [g L^{-1}]			Dextrins [g L^{-1}]
		glucose	maltose	maltotriose	
With hop acids	4±0.1 ^{a}	21.2±0.6 ^{b}	0.3±0.1 ^{a}	0.1±0.1 ^{a}	150.6±2.8 ^{a}
	5±0.1 ^{a}	39.5±1.1 ^{d}	0.4±0.1 ^{a}	0.5±0.1 ^{a}	143.8±2.7 ^{a}
Without hop acids	4±0.1 ^{a}	18.9±0.5 ^{a}	0.3±0.1 ^{a}	n.d.	149.1±2.8 ^{a}
	5±0.1 ^{a}	29.8±0.8 ^{c}	0.5±0.1 ^{a}	0.2±0.1 ^{a}	147.6±2.9 ^{a}

n.d. – not detected; different superscript letters in columns indicate significant differences (P <0.05) between mean values.

Table 3: Chemical composition of fermented mashes.

Mashes after fermentation	initial pH	pH after 72 h	Concentration of sugars [g L^{-1}]			Dextrins [g L^{-1}]	Ethanol [% v v^{-1}]
			glucose	maltose	maltotriose		
With hop acids	4±0.1 ^{a}	3.8±0.2 ^{a}	0.1±0.1 ^{a}	n.d.	0.3±0.1 ^{b}	0.9±0.1 ^{a}	11.7±0.6 ^{b}
	5±0.1 ^{a}	4.4.±0.1 ^{b}	0.2±0.1 ^{a}	0.1±0.1 ^{a}	0.1±0.1 ^{a}	1.9±0.1 ^{c}	9.6±0.5 ^{a}
Without hop acids	4±0.1 ^{a}	3.5±0.1 ^{a}	0.2±0.1 ^{a}	1.0±0.1 ^{b}	0.1±0.1 ^{a}	0.9±0.1 ^{a}	10.3±0.5 ^{a}
	5±0.1 ^{a}	4.2±0.1 ^{b}	0.1±0.1 ^{a}	0.2±0.1 ^{a}	0.1±0.1 ^{a}	1.6±0.1 ^{b}	9.5±0.5 ^{a}

n.d. – not detected; different superscript letters in columns indicate significant differences (P <0.05) between mean values.

In mashes before fermentation, the concentration of glucose was higher in samples with initial pH 5. As mentioned above, the acid α-amylase reveal a higher activity at pH 5 than at pH 4. During 'activation' of starch, glucose was the most liberated sugar, followed by maltose, and the lowest in maltotriose, for both pH variants. It is mainly caused by the enzyme mechanism. Despite significant differences in concentrations of sugars determined upon completion of fermentation, it can be observed that all mashes, regardless of the starting pH, have been fermented properly. The lowest concentrations of unutilized glucose, maltose, maltotriose were determined in mashes supplemented with hop α-acid preparation, with initial pH 4 (Table 3). Taken into consideration the dextrin content in mashes, it can be suppose that the saccharification enzymes did not manage to break them down to the simple sugars available for yeast. The lowest dextrin content in the fermented mash with initial pH 4, and supplemented with hop α-acids preparation is reflected in the highest ethanol content (11.7±0.6% v v^{-1}) with comparison to the remaining variant.

Sweet mashes and mashes during fermentation were examined microbiologically. The results obtained for samples collected once every 24 h are shown in Figures 2 and 3.

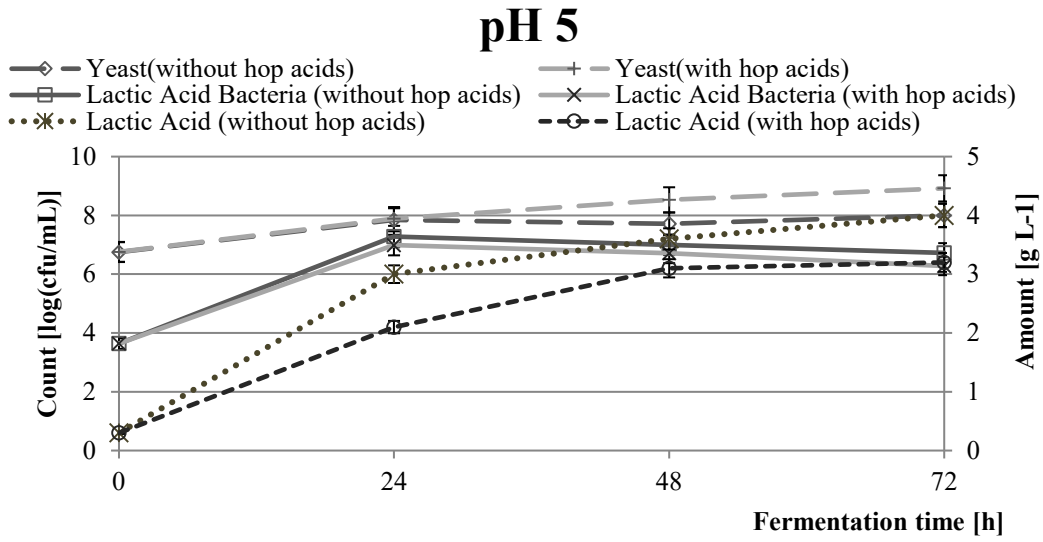

Figure 2 Microbiological analysis of mashes with initial pH 5, during fermentation.

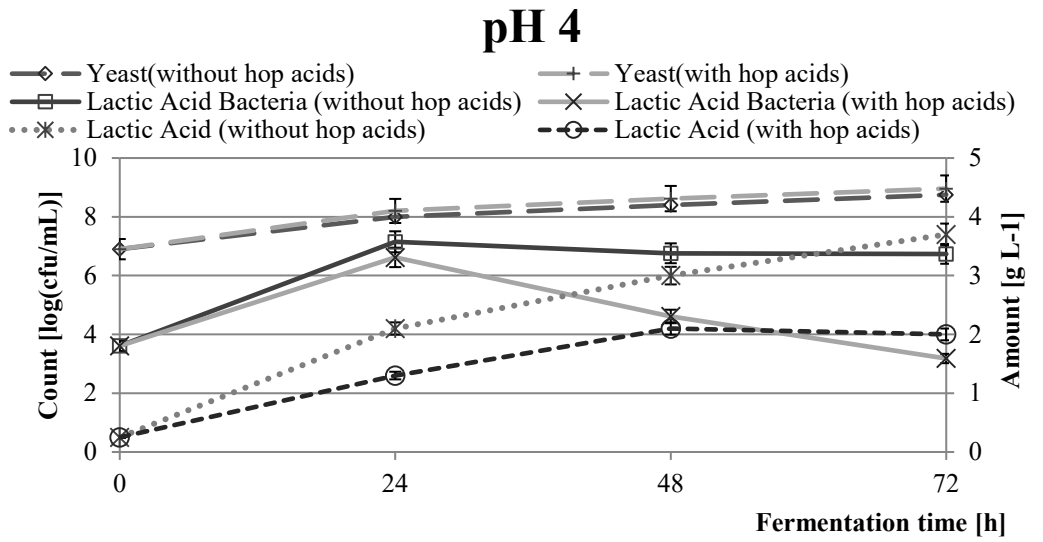

Figure 3 Microbiological analysis of mashes with initial pH 4, during fermentation.

Furthermore, during fermentation, lactic acid content was determined in the mashes (Figs. 2 and 3). The main sources of the microbial contaminations of distillery mashes are raw materials, yeast, equipment, water, and air. This is a serious problem for producers of raw spirit. Yeast and lactic acid bacteria are often found together in natural ecosystems and can compete for the same nutrients. When both microorganisms develop and live together in a specific medium, in which yeast growth is limited by providing suboptimal vitamin concentrations, missing substances (including nicotinic acid, adenine, guanine, aspartic acid, tryptophan, glycine, alanine or lysine) necessary for growth *Lactobacillus* spp. are synthesized in the medium by yeast cells (Narendranath, Hynes, Thomas, & Ingledew, 1997). The obtained results indicated a decrease in pH from 5 to 4, which limits the growth of undesirable lactic bacteria. It was observed that regardless of the initial pH of the mashes, the number of lactic bacteria cells in the medium before the fermentation was at a similar level of 3.63±0.79 log CFU mL^{-1}, while the concentration of lactic acid in mashes reached 0.03±0.01 g L^{-1}. After 24 hours of fermentation in reference mashes(without the addition of hop α-acids preparation), more than 2-fold increase in the number of bacteria cells was noted, and in samples with initial pH 4 reached 7.15±0.29 log CFU mL^{-1}, while for those with pH 5 amounted

7.82±0.32 log CFU mL^{-1}. The addition of hop α-acids preparation to the mashes with the initial pH 4.0 allowed to reduce the number of lactic acid bacteria after 72 h of the process to 3.18±0.1 log CFU mL^{-1}, whereas in the mashes with the initial pH 5.0, lactic acid bacteria count was 6.28±0.2 log CFU mL^{-1}. Also, the content of lactic acid, in comparison to the control samples (without hop α-acids), decreased by 1.7 g L^{-1} for pH 4, and by 0.44 g L^{-1} for pH 5. The count of bacteria decreased with the increase in the final lactic acid concentration, when lactic acid together with ethanol were present in the mashes. This suggests that ethanol acts synergistically with lactic acid to kill these bacteria and that the toxicity of ethanol is increased by the drop in pH caused by lactic acid in the medium.

Suzuki (2011) stated that hop acids affect not only the exhaustion of proton strength and by the capture of divalent cations, such as $Mn2^{+.}$ They disrupt the enzymatic processes of proteins involved in energy production and redox mesostase in a bacterial cell.

The disturbance of the membrane mechanism, cellular processes, and intracellular acidification results in inhibition of the active transport of sugars, nutrients and amino acids through the membrane, and thus interruption of the respiration and synthesis of protein, DNA and RNA, ultimately cell death leading to its death (Doyle & Roman, 2016).

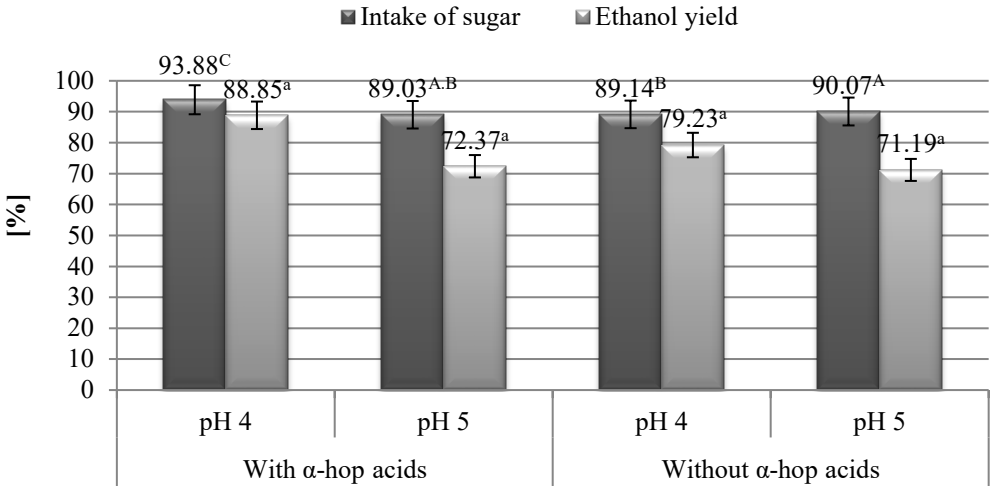

Figure 4 Ethanol fermentation factors.

Different superscript capital letters indicate significant differences (P <0.05) between mean values of sugars intake.
Different superscript lowercase letters indicate significant differences (P <0.05) between mean values of ethanol yield.

The activity of lactic acids has been shown to decrease the yeast growth rate, sugar consumption, and ethanol yield (Strąk & Balcerek, 2015; Thomas, Hynes, & Ingledew, 2001). Lower ethanol contents and fermentation efficiency were observed in trials exhibiting more severe bacterial contamination (Tabs. 2,3 and Figs. 2-4).

Only a 1% drop in ethanol yield is of great importance for food alcohol distillers because their profit margin is very narrow (Makanjuola, Tymon, & Springham, 1992). In large plants with capacities from 400 million to 1100 million liters of ethanol per year, such a decrease would reduce income by 1 million to 3 million per year (Narendranath et al., 1997). Research on the direct effects of process contamination is not easy to carry out (Makanjuola et al., 1992).

Estimation of the ethanol fermentation factors showed that the lower pH of the mashes (at the level of 4), and the use of α-hop acids preparation allowed to improve the efficiency of the process by 5% comparing to the control sample. On the other hand, raising the pH to 5, resulted in a 5% decrease in the yield, despite the use of hop acids preparation. In all tested mashes, the intake of sugars by yeast was at a similar level (Fig. 4).

Conclusion

Bacterial contamination of the fermentation medium is the main cause of the reduction of ethanol yield during the fermentation of starchy raw materials. Among the bacterial contaminants encountered, lactic acid bacteria are the

most troublesome, because of their tolerance to high temperature and low pH and the ability to grow quickly. The obtained results indicate that the use of hop α-acids preparation allows reducing the development of microbial infection, which are mainly lactic bacteria. Also, it allows an ethanol yield to be improved. Taking into consideration, that the starchy raw materials, among others cereal grains, may include contaminating bacteria, which compete with the yeast on growth-promoting nutrients, there is a need to apply addition to distillery mashes of antimicrobial preparations, to improve production technologies and obtain products of the highest quality.

References

Agu, R. C., Swanston, J. S., Walker, J. W., Pearson, S. Y., Bringhurst, T. A., Brosnan, J. M., & Jack, F. R. (2012). Predicting Alcohol Yield from UK Soft Winter Wheat for Grain Distilling: Combined Influence of Hardness and Nitrogen Measurements. *Journal of the Institute of Brewing*, *115*(3), 183–190. https://doi.org/10.1002/j.2050-0416.2009.tb00367.x

AOAC. (1995). Official Methods of Analysis of AOAC International (16th ed.). Meryland: AOAC International.

Arshad, M., Zia, M. A., Asghar, M., & Bhatti, H. (2011). Improving bio-ethanol yield : Using virginiamycin and sodium flouride at a Pakistani distillery, *10*(53), 11071–11074. https://doi.org/10.5897/AJB09.1273

Bischoff, K. M., Skinner-Nemec, K. A., & Leathers, T. D. (2007). Antimicrobial susceptibility of Lactobacillus species isolated from commercial ethanol plants. *Journal of Industrial Microbiology & Biotechnology*, *34*(11), 739–744. https://doi.org/10.1007/s10295-007-0250-4

Bringhurst, T. A., & Brosnan, J. (2014). *Scotch whisky: raw material selection and processing*. *Whisky* (Second Edi). Elsevier Ltd. https://doi.org/10.1016/b978-0-12-401735-1.00006-4

BS EN ISO 10520:1998. Native starch. Determination of starch content. Ewers polarimetric method (1998).

Doyle, M. P., & Roman, D. J. (2016). Growth and Survival of Campylobacter fetus subsp. jejuni as a Function of Temperature and pH. *Journal of Food Protection*, *44*(8), 596–601. https://doi.org/10.4315/0362-028x-44.8.596

Essia Ngang, J. J., Letourneau, F., & Villa, P. (1989). Alcoholic fermentation of beet molasses: effects of lactic acid on yeast fermentation parameters. *Applied Microbiology Biotechnology*, *31*, 125–128.

Gibson, B. R., Lawrence, S. J., Leclaire, J. P. R., Powell, C. D., & Smart, K. A. (2007). *Yeast responses to stresses associated with industrial brewery handling*. https://doi.org/10.1111/j.1574-6976.2007.00076.x

HGCA, A. (2018). Cereals and Oilseeds Division of the Agriculture and Horticulture Development Board (AHDB). Retrieved October 17, 2018, from https://cereals.ahdb.org.uk

Hulda, Y., Njintang, Y. N., & Cmf, M. (2017). Optimization of the use of Betterave (Beta Vulgaris) for the Production of Green Energy. *Global Journal of Science Frontier Researche*, *17*(2).

Islam, M., Toledo, R., & Hamdy, M. K. (1999). Stability of virginiamycin and penicillin during alcohol fermentation, *17*, 369–376.

Makanjuola, D. B., Tymon, A., & Springham, D. G. (1992). Some effects of lactic acid bacteria on laboratory-scale yeast fermentations. *Enzyme and Microbial Technology*, *14*, 350–357. https://doi.org/10.1016/0141-0229(92)90002-6

Narendranath, N. V., Hynes, S. H., Thomas, K. C., & Ingledew, W. M. (1997). Effects of lactobacilli on yeast-catalyzed ethanol fermentations. *Applied and Environmental Microbiology*, *63*(11), 4158–4163.

Oliva-Neto, P., & Yokoya, F. (1994). Evaluation of bacterial contamination in a fed-batch alcoholic fermentation process. *World Journal of Microbiology & Biotechnology*, *10*, 697–699.

Pietruszka, M., & Szopa, J. S. (2014). Agricultural Distillates from Polish Varieties of Rye, *32*(4), 406–411.

Pomeranz, Y., & Meloan, C. E. (1995). *Carbohydrates. Food Analysis* (3rd ed.). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-6998-5

Ralph, R. J. (1981). Practical aspects of operating an alcohol plant. In T. P. L. (ed.) (Ed.), *A step to energy independence—a textbook for fuel alcohol production.* (pp. 255–265). Lexington, Ky: Alltech Technical Publications.

Reed, G., & Nagodawithana, T. W. (1991). *Yeast Technology* (Seckond Ed). New York. https://doi.org/10.1007/978-94-011-9771-7

Rückle, L., & Senn, T. (2006). Hop acids as natural antibacterials can efficiently replace antibiotics in ethanol production. *International Sugar Journal*, *108*(1287), 139–147.

Schell, D. J., Dowe, N., Ibsen, K. N., Riley, C. J., Ruth, M. F., & Lumpkin, R. E. (2007). Contaminant occurrence, identification and control in a pilot-scale corn fiber to ethanol conversion process. *Bioresource Technology*, *98*(15), 2942–2948. https://doi.org/10.1016/j.biortech.2006.10.002

Simpson, W. J., & Smith, A. R. W. (1992). Factors affecting antibacterial activity of hop compounds and their derivatives, 327–334.

Skinner, K. A., & Leathers, T. D. (2004). Bacterial contaminants of fuel ethanol production. *Journal of Industrial Microbiology & Biotechnology*, *31*(9), 401–408. https://doi.org/10.1007/s10295-004-0159-0

Strąk-Graczyk, E., & Balcerek, M. (2019). Simultaneous saccharification and fermentation of native rye , wheat and triticale starch a * Maria Balcerek , a Katarzyna Przybylsk a. *Journal of Sci Ence and Food*

Agriculture, *99*(May), 4904–4912. https://doi.org/10.1002/jsfa.9718
Strąk, E., & Balcerek, M. (2015). Wybrane technologie wykorzystywane w przemyśle gorzelniczym. *Acta Scientiarum Polonorum, Biotechnologia*, *14*(1), 33–44. Retrieved from http://www.acta.media.pl/pl/main.php?s=3&no=506&p=21&id=4451&lang=pl
Suzuki, K., Iijima, K., Sakamoto, K., Sami, M., & Yamashita, H. (2006). A Review of Hop Resistance in Beer Spoilage Lactic Acid Bacteria.
Thomas, K. C., Hynes, S. H., & Ingledew, W. M. (2001). Effect of lactobacilli on yeast growth, viability and batch and semi-continuous alcoholic fermentation of corn mash. *Journal of Applied Microbiology*, *90*(5), 819–828. https://doi.org/10.1046/j.1365-2672.2001.01311.x
Verzele, M., & De Keukeleire, D. (1991). Chemistry and analysis of hop and beer bitter acids. *Developments in food science*, *27*(169), 417.
Villegas-Torres, M. F., Ward, J. M., & Lye, G. J. (2015). The protein fraction from wheat-based dried distiller's grain with solubles (DDGS): Extraction and valorization. *New Biotechnology*, *32*(6), 606–611. https://doi.org/10.1016/j.nbt.2015.01.007
Walker, G. M., & Hill, A. E. (2016). Saccharomyces cerevisiae in the Production of Whisk (e) y, (i), 1–15. https://doi.org/10.3390/beverages2040038

