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Non-alcoholic beer production – an overview

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Through years beer became one of the best known alcoholic beverages in the world. For some reason e.g. healthy lifestyle, medical reasons, driver's duties, etc. there is a need for soft drink with similar organoleptic properties as standard beer. There are two major approaches to obtain such product. First is to interfere with biological aspects of beer production technology like changes in mashing regime or to perform fermentation in conditions that promote lower alcohol production or using special (often genetic modified) microorganism. Second approach is to remove alcohol from standard beer. It is mainly possible due to evaporation techniques and membrane ones. All these approaches are presented in the paper.

Keywords: non-alcoholic beer, fermentation, membrane separation, dealcoholisation, evaporation.

INTRODUCTION

Beer is one of the oldest known beverages in human history. The eldest known document is a clay plate from Sumer civilisation that shows process of beer production and it was estimated to be about 6000 years old[1]. Beer was widely known in ancient times and played a significant role in civilisations of that era. For instance in Babylon in Hammurabi's law there are few paragraphs according to beer and its production[2]. In Egypt beer was also very popular, what's more in Nekhen (also known as Hieraconpolis) archaeologists found the oldest brewery in the world preserved to our times[3]. In years 1998–2007 Polish archaeologists led by prof. Krzysztof Ciałowicz from Jagiellonian University found in Tell el-Farcha a big brewery complex containing of six breweries similar to that discovered in Nekhen but about one century younger. Still there are assumptions that those breweries were built on the fundaments of older ones[4]. From XII century hops become more popular and finally in 1516 German purity law defined beer as a drink made of water, barley and hops (yeasts were unknown in XVI century)[5, 6]. From that time there were no significant changes in brewery until XIX century when science became interested in that process[7].

Throughout the history numerous researchers and physicians were interested in influence of food and drinks to the human health. Rejection of beer was diagnosed as illness[8]. In ancient times beer was also used as a medicine or as a solvent for extraction of compounds present in plant matter[9, 10]. As far we know about that types of beer-based treatment for snakes bite, internal pain, ear infection and problems with urinary tract. Beer without additional ingredients was used as a promoter of defecation and urination. Anaesthetic and antitussive effects of that drink were also known[11].

Our present knowledge about beer is far more complex than in ancient times. It is known that beer is a complex mixture including not only alcohol but many other compounds also and plenty of them affect human organism. In standard lager beer the amount of ethanol is about 4–5% of volume. In our organisms liver is one of the organs responsible for removing harmful substances from our body. Ethanol is one of those irritants but in larger amounts it affects liver and causes another alcoholic diseases[12–14]. Ethyl alcohol is linked also with breast, liver and colorectal cancers. Carcinogenicity of ethanol is probably caused by acetaldehyde, by-product in alcohol metabolism. This compound may damage DNA. Furthermore ethanol is accused for being a solvent for other carcinogens and for producing reactive oxygen species[15].

However, beer is not only an alcoholic drink but it contains many other compounds that affect human body. Plenty of them are delivered by hops (*Humulus lupus*). The most know are the properties of xantohumol and isoxantohumol. These are prenylflavonoids that counteract cancer growth by inhibition of procarcinogens activation and induction of enzymes responsible for carcinogen detoxification. Another xantohumol features are antioxidant and estrogenic activity[16]. Isoxantohumol shows anti-viral properties and can prevent dementia and reduce risk of obesity-induced cognitive decline[17, 18].

By the removal of alcohol from beer it is possible to get isotonic drink with significant influence to health. For non-alcoholic beer the level of ethanol should be low, but may differ in various countries even in the European Union. In Poland non-alcoholic beer shouldn't contain, similarly to fermented milk products[19], no more than 0.5% of volume[20]. In Germany, USA and in China this limit is the same as in Poland, but in Spain maximal volume of alcohol is 1% while and in France 1.2% of volume[21].

There are two ways to lower the level of ethanol in beer. First is to interfere with the biology of brewing to prevent formation of alcohol during the fermentation. Second one is to remove alcohol from a standard alcoholic beer. Both of those approaches are discussed in further paragraphs.

BIOLOGICAL APPROACH TO OBTAIN LOW ALCOHOL CONTAIN IN BEER

a) Changes in mashing regime

Beer production starts with grinding the grain in order to ensure extraction of malt sugars and enzymes responsible for sugar bonds hydrolysis to the fermentation broth. In standard procedure to provide optimal conditions for all enzymes the mashing process is conducted through

wide spectre of temperatures from about 60°C to nearly 80°C. The most important enzymes that deliver sugars for fermentation are:

– β-glucanase – the enzyme responsible for degradation of polysaccharides connected by β-glycoside bonds. Such bonds are present in cellulose from malt grains. This type of enzyme represents the highest efficiency at 40–45°C.

– β-amylase – the role of this enzyme is to cleave α-1,4-glycoside bonds present in starch, releasing maltose molecules from non-reducing end of starch chains. Optimal temperature for this enzyme is in range between 60–65°C.

– α-amylase – this enzyme cleaves α-1,4-glycosidic bonds located in the middle of a starch chain. This enzyme has wide spectrum of optimal temperatures between 65–75°C[22].

As it was written above during the mashing process various enzymes are active in different temperatures. The aim of this approach is to prepare wort containing less fermentable sugars than in standard one. Lowering the level of fermentable sugars decreases formation of alcohol by yeasts. There are few ways to achieve that target.

By application high temperature (75–80°C) during mashing process, only α-amylase is active. Even though this range of temperatures isn't optimal for that enzyme, it is active enough to digest the starch into smaller parts. But due to the inactivation of β-amylase, the products of α-amylase activity cannot be converted into maltose being fermentable by yeasts. In general opinion the flavour of beer produced by this way is very good, although there are some reports of a malty taste of such product[24, 25].

Another approach is called cold water malt extraction. This procedure allows to capture color and flavour from malts while limiting the extraction of complex carbohydrates. Temperature during that process is lower than 60°C. These conditions also prevent increasing wort gravity during the mashing process[25, 26].

Both of presented methods can be combined. At the beginning of that process malt is mashed under low temperature as in cold water malt extraction. Next the whole mixture is heated up to high temperature above 75°C. Recent studies on that type of infusion mashing showed that beer produced by 30 minutes of cold water extraction and 120 minutes of mashing at 77°C had wort extract at the level of 8.5% and alcohol content 0.44% of volume. This level of ethanol is fully acceptable for non-alcoholic beers[26].

Spent grains remaining after standard beer production procedure contain between 40–50% polysaccharides in dry mass[27]. The spent grains may be re-mashed to recover these carbohydrates and use to non-alcoholic beer production. It is possible due to the low level of fermentable sugars present in remashing product. Efficiency of the process may be increased by pre-treatment of grains using enzymes such as cellulases and glucanases. The potential advantage of this method is production of two kinds of beer from one dose of grain[28].

b) Changes in fermentation process

After brewing process, wort is boiled to denaturate enzymes and another proteins. During this phase hop is added to provide bitterness and aroma and in the next step the mixture is cooled down before yeasts addition and fermentation start. In standard process yeast are responsible for production of ethanol and another compounds responsible for aroma and taste of beer.

Researchers working on non-alcoholic beer production methods tried to interfere with yeast metabolism to limit the alcohol fermentation process. It is possible by changing the fermentation conditions or by interrupting the process by partial yeasts inactivation. On the other hand process may also be changed through the immobilisation of yeasts. *Saccharomyces cerevisiae* may be replaced by other strains or even by different species.

One of the easiest way to obtain changes in yeast metabolism is cold contact process. During this method elaborated by Schur in 1983 wort is cooled down to about 1°C and pH is kept at about 4.0[29]. The aim of this approach is to lower the rate of ethanol formation without significant decrease in production of esters and higher alcohols. 3-methylbutanal, 2-methylbutanal and 3-methylthiopropionaldehyde formed during this process are responsible for characteristic flavour of non-alcoholic beer[30].

Another approach is called an arrested fermentation. During this process yeasts are inactivated or removed before they start producing ethanol in large amounts. It is done usually by rapid cooling down the fermenting beer to 0°C, pasteurization or centrifugation[31]. Recent studies showed that inactivation process may be also performed by microbubbled carbon dioxide at low pressure. What's more the process has minimal influence on the final product taste[32]. The main difficulty using this method is to choose the right moment to stop fermentation. Self-aggregation of *Saccharomyces cerevisiae* cells can be useful to easy separation. Yeast flocules have macroscopic size and they naturally sediment during the process[33, 34].

Beers produced in described way represent similar flavour as beers made using cold contact process. One of the methods to minimize that effect is using selected yeast strains for instance those overproducing higher alcohols like isobutanol and isoamyl alcohol[35].

Immobilisation of microorganisms is popular and widely known in numerous biotechnology processes which allows for carrying out continuous process with accurate calculation and control the residence time of the process[36, 37]. Immobilization allows for shortening the time of process, reducing costs and obtaining different products. Carrier for the immobilisation should be non-toxic, affordable and allow for high yeast cell concentration[38, 39].

There is also a possibility to concentrate yeast cells in membrane bioreactor in which the process can run faster and yeast concentration is controlled and regulated in time[40–42]. Recent studies showed that increase in mass of immobilised cells caused the reduction of fermentation time but not whole process. It is caused by synthesis of larger amount of carbonyl compounds[43]. This feature may be useful in non-alcoholic beer production.

Production of non-alcoholic beers using immobilisation of yeast cells could be a feasible approach for industrial scale. It is known that one alcohol free beer installation based on packed bed immobilized yeast bioreactor is operating in Netherlands in brewery Bavaria[44, 45].

The last biological method for alcohol free beer obtaining is based on genetic modifications of *Saccharomyces cerevisiae* genus or by using another microorganisms for

fermentation. Recent studies showed that *Sacharomyces cerevisiae* strain isolated from cachaça distilleries in Brazil produces more flavour compounds than standard one. Thus it may be more useful in production of non-alcoholic beers that often represent lack of aroma[46, 47]. There were also attempts to use brewing yeasts mutants with deficiency in tricarboxylic acid cycle enzymes. The effect of such experiment was a beer with concentration of alcohol between 0.07 and 0.31% of volume. From tested yeasts the best were those with lack of 2-oxoglutarate dehydrogenase activity and fumarase activity. Other mutants represented significant levels of produced diacetyl that gives unpleasant buttery flavour in beer[48].

Lower amount of ethanol is obtained also with *Zygosaccharomyces rouxii*[49]. Additionally, this strain is able to consume ethanol under aerobic conditions[25]. *Saccharomycodes ludwigii* strains named as DBVPG 3010 isolated from grape produced 0.5% of ethanol and appreciable amount of esters[50]. Other species used for non-alcoholic beer production were *Kluyveromyces bulgaricus and fragilis, Scheffersomyces shehatae*, *Wickerhamomyces anomalus Pichia kluyveri*[51–53].

Thus these compounds should be supplemented after alcohol removing[56]. The main advantage of this method is the possibility to obtain the level of ethanol lower than 0.5% of volume. This process is also affordable and simple to perform[57].

Another method to separate compounds due to their difference of volatility is spinning cone column. In such device desorption of low-boiling compounds from thin film of liquid is performed. The installation is similar to a centrifuge. It consists of two series of inverted cones, one attached to the inside wall of the column and the second one to the rotating shaft. Beer is delivered from the top of spinning cone column. It is pulled down by gravity on the surface of first fixed cone and then dropped on the first cone attached to the rotating shaft. Due to the centrifugal force beer flows as a thin film upward to the rim of the cone and fails down on the second stationary cone. The stripping medium is a steam delivered from the bottom of the installation. The vapour flows out through the top of the column into condenser. Dealcoholized beer is collected at the bottom of the spinning cone column[58]. Mentioned process can be carried out in continuous system also at residence time about 20s and

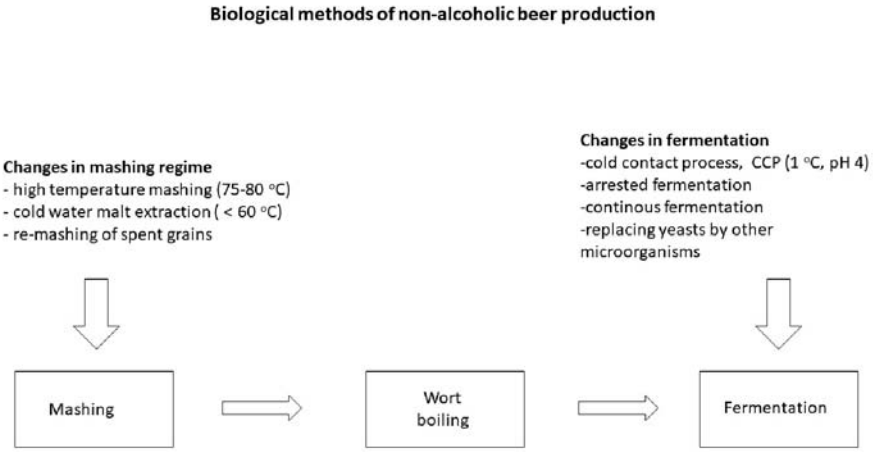

Figure 1. Scheme of biological methods used for non-alcoholic beer production and points in the brewing process in which they are applied

All presented biological methods are schematically presented in Fig. 1.

PHYSICAL APPROACH TO OBTAIN LOW ALCOHOL CONTAIN IN BEER

In this approach the goal is to remove ethanol from standard beer without losing other compounds responsible for the flavour and taste of the product. There are two major possibilities to remove alcohol from a typical beer. First is to evaporate ethanol. Second one is to separate alcohol from a beer using membrane techniques.

a) Thermal methods for beer dealcoholisation

Rectification is one of the easiest methods to separate volatile compounds. In this approach the mixture is being distilled to fractionate its elements due to their boiling point. Ethanol's boiling point is 78°C[54]. This temperature may be lowered with lowering the pressure. Unfortunately, simultaneously with alcohol some volatile compounds that have similar boiling point to ethanol are also removed[55] as well as carbon dioxide. temperature 40–55°C[59]. Recent studies showed that it is possible to remove more than 94% of alcohol present in beer[60]. The major disadvantage of such process is high possibility of oxygen input[61] however spinning cone columns and other similar thin film evaporators seem promising for removal of alcohol on industrial scale.

b) Membrane methods for beer dealcoholisation

The simplest membrane process for alcohol removal is dialysis. The driving force of this method is a concentration gradient of each compound across the semipermeable membrane. Small molecules can traverse membrane while the bigger than membranes pores not. In such conditions alcohol and other small molecule compounds transfer across the membrane from higher concentration (beer) to water[62]. The main advantage of dialysis is low operating temperature and possibility to remove alcohol to the acceptable level of 0.5% of volume[57]. The biggest disadvantage is loosing some compounds responsible for colour, taste and aroma and generation a huge amount of very diluted alcohol in receiving solution[63, 64].

Reverse osmosis is a process in which dissolved substances are separated using semi permeable membrane. The basis of this process is to create higher transmembrane pressure than osmotic pressure of the solution[65]. Reverse osmosis is a well-known and applicable process. Such installations are widely used in water treatment and water desalination modules[66, 67]. They are already in use in food and beverage industry e.g. for juice concentration. Studies showed that there is a possibility to remove alcohol to level lower than 0.5% of volume at low operating temperatures (20°C)[68, 69]. Similarly to another separation processes significant loses in esters and higher alcohols responsible for beer features were noticed[70]. Reverse osmosis seems to be feasible method for dealcoholisation of beer especially with invention of new types of membranes that have better selectivity for ethanol unlike other ingredients. It should be noten that before direction the stream to reverse osmosis unit, the microbial bacteria should be separated using classical filtration or micro, – ultrafiltration[71, 72].

Pervaporation is another membrane approach in which certain, volatile compounds preferentially permeate through the membrane and evaporate downstream. The vapor may be released or condensed and collected. The driving force of pervaporation process is the chemical potential gradient across the membrane and it can be increased using vacuum pump or inert gas purge[73, 74]. As far this separation method is being used in many fields like desalination of water, separation of organic-organic mixtures or in recovery of waste industrial solvents[75–77]. The main advantages of pervaporation are low energy consumption and no vapour-liquid equilibrium limits. Running conditions are easy to obtain and don't have negative influence on a beer itself. Temperature for that process is about 50°C[78]. Alcohol content may be reduced to 0.6% of volume what requires combination of that method with another one to obtain non-alcoholic beer accepted in most of the countries[21]. However pervaporation gives the opportunity to achieve beer that have nearly the same aroma profile as the original beverage[79, 80] that is a huge advantage to another separation methods.

Another membrane process for alcohol removal is osmotic distillation also called isothermal membrane distillation. This process is very often applied instead classical distillation when high temperature is not recommended[81]. The driving force of this process is vapour pressure difference across microporous, hydrophobic membrane. Stripping solution flows in counter-current mode removing alcohol that passes through the membrane.

In dealcoholisation ethanol evaporates at the membrane pores then its vapour diffuses through the membrane pores and finally ethanol condensates in the stripping medium[82, 83]. As a stripping solution can be used water and most often concentrated salt solutions[84, 85]. In the second case some amount of salt can diffuse into beer. An interesting solution is the use of glicerol as a stripping solution[86]. Studies showed that during osmotic distillation there were not significant loses in nutrients like maltose or glycerol[87], however significant in volatile compounds (77% of initial level). Esters and higher alcohols responsible for aroma profile of a beer were lost in 90%[88].

All presented physical methods are schematically presented in Fig. 2.

CONCLUSION

In the near future the market of non-alcoholic beers will probably increase. Trends in a healthy lifestyle have caused a decline of alcohol consumption in Europe[89]. Today non-alcoholic beers become more and more popular. In 2017 market of non-alcoholic beers in Poland increased over 20%[90]. The most popular methods of dealcoholisation are arrested fermentation and distillation but beer produced on this way has worse taste and aroma than regular beer[91].

After analysis available methods it looks that the best way to obtain good quality non-alcoholic beer is a combination of biological methods with physical ones. In our future research we are going to put much more attention to control an ethanol yield coefficient obtained at different cells concentration. The process will be carried out in membrane bioreactor in which the cells concentration is easy to control by particular streams regulation[40]. Permeate obtained from this reactor could be directed directly for ethanol separation and recovery. Pervaporation process looks the most promising, in which there is a possibility to achieve beer with same aroma and taste as the original beverage. Market

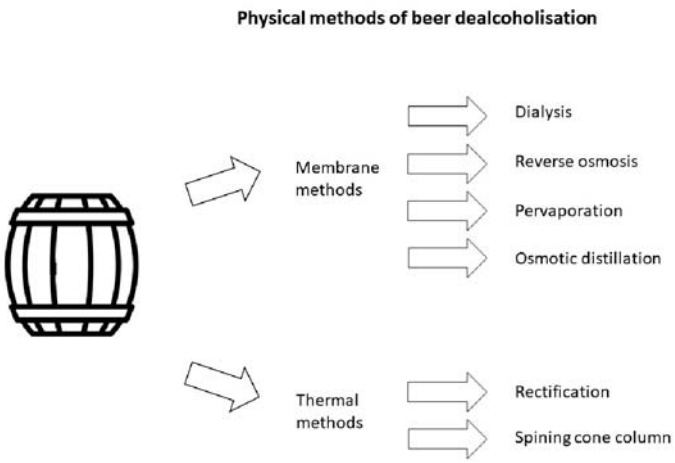

Figure 2. Scheme of physical methods used in beer dealcoholisation

of membrane production for pervaporation process still is growing up what promises well for achieving success in this area of research.

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