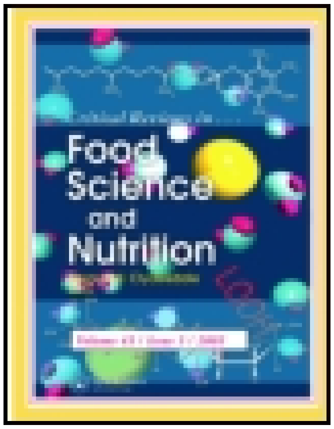

Critical Reviews in Food Science and Nutrition

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Low-alcohol beers: Flavour compounds, defects and improvement strategies

Carlos A. Blanco [a] , Cristina Andrés-Iglesias [b] & Olimpio Monero [b]

[a] Dpto. Ingeniería Agrícola y Forestal (Área de Tecnología de los Alimentos), E.T.S. Ingenierías Agrarias , Universidad de Valladolid , 34004 , Palencia , Spain

[b] Centre for Biotechnology Development (CDB), CSIC. Boecillo's Technological Park , Av. Francisco Vallés, 8. 47151—Boecillo, Valladolid , Spain

Accepted author version posted online: 13 Aug 2014.

Low-alcohol beers: Flavour compounds, defects and improvement strategies

By

Carlos A. Blanco, Cristina Andrés-Iglesias and Olimpio Monero*[1]

Dpto. Ingeniería Agrícola y Forestal (Área de Tecnología de los Alimentos)

E.T.S. Ingenierías Agrarias. Universidad de Valladolid,

34004 Palencia, Spain

(1) Centre for Biotechnology Development (CDB), CSIC. Boecillo's Technological Park, Av. Francisco Vallés, 8. 47151 – Boecillo, Valladolid, Spain

*To whom correspondence should be addressed

e-mail: cblanco@iaf.uva.es

FAX: 3479108302;

Abstract

Beer consumers are accustomed to a product that offers a pleasant and well-defined taste. However, in alcohol-free and alcohol-reduced beers these characteristics are totally different from those in regular beer. Therefore, it is important to evaluate and determine the different flavour compounds that affect organoleptic characteristics to obtain a product that does not contain off-flavours, or taste of grass or wort. The taste defects in alcohol-free beer are mainly attributed to loss of aromatic esters, insufficient aldehydes, reduction or loss of different alcohols, and an indeterminate change in any of its compounds during the dealcoholization process. The dealcoholization processes that are commonly used to reduce the alcohol content in beer are shown, as well as the negative consequences of these processes to beer flavour. Possible strategies to circumvent such negative consequences are suggested.

Keywords: beer, dealcoholization, taste, off-flavours.

INTRODUCTION

Beer is a beverage brewed principally from malt, hops and water, and the mixture is fermented by using yeast. It is one of the most popular drinks worldwide (Lehnert et al., 2008), its popularity arising from its pleasant sensory attributes, together with favourable nutritional characteristics for light-to-moderate consumption (Sohrabvandi et al., 2010).

Low-alcohol beer is a beer with very low or no alcohol content. The alcohol by volume (ABV) limits depend on laws in different countries. In most of the EU countries beers with low alcohol content are divided into alcohol free beers (AFBs) less than or equal to 0.5% (ABV) and low-alcohol beers (LABs) with no more than 1.2% ABV. In the United States alcohol-free beer means that there is no alcohol present, while the upper limit of 0.5% ABV corresponds to so-called non-alcoholic beer or ‘‘near-beer’’ (Brányik et al., 2012).

Although it is still a minor product of the brewing industry, the increasing production of low-alcohol beers worldwide reflects the global trend for a healthier lifestyle (Lehnert et al., 2009) and civil reasons (Catarino, et al., 2009). Alcohol-free beers are recommended for specific groups of people such as pregnant women, sporting professionals, people with cardiovascular and hepatic pathologies, and people on medication. (Sohrabvandi et al., 2010; García et al., 2004). On the other hand, the market for non-alcoholic brews has experienced an increase over the last five-to-ten years, mainly because of new drink/driving rules, health and religious concerns (Catarino and Mendes, 2011; Sohrabvandi et al., 2010; Caluwaerts, 1995). However, some of the low-alcohol beers that are commercially available are not popular with consumers because of their lack of aroma and flavour (compounds) (Catarino et al., 2009).

At present, there are several methods for the production of low-alcohol beers (Sohrabvandi et al., 2010; Brányik et al., 2012):

a) To remove the ethanol from a completely fermented beverage by using several separation processes. The most common separation processes used for beverages dealcoholization are heat treatment and membrane-based processes (Catarino et al., 2007). Heat treatment processes comprise of evaporation and distillation or steam stripping, both under vacuum conditions (Belisario-Sánchez et al., 2009). Membrane based processes include reverse osmosis, nanofiltration, dialysis and pervaporation (Labanda et al., 2009). The industrial methods widely applied for beer dealcoholization are vacuum evaporation, vacuum distillation, dialysis and reverse osmosis (Brányik et al., 2012). Removal of alcohol from regular beer using processes that encompass extreme conditions such as distillation or evaporation can cause the loss of the original aroma (owing to chemical and physical reactions) (Lehnert et al., 2009; Catarino et al., 2009).

b) To control alcohol formation during brewing (Lehnert et al., 2009). This can be achieved by either restricting ethanol formation or shortening the fermentation process. Obtaining low alcohol content via interrupted fermentation is accompanied by low contents of aroma and flavour compounds. In order to avoid these shortcomings processes have been developed for low ethanol production that involve the use of special or immobilized yeasts as well the use of low sugar raw materials (Catarino and Mendes, 2011).

Hence, the standing issue in the production of low-alcohol beers in terms of organoleptic characteristics is the achievement of a product ‘as close as possible’ to regular beer (Sohrabvandi

ACCEPTED MANUSCRIPT

et al., 2010). Beer flavour results from a mixture of by-products formed during yeast growth phases that match up to metabolic pathways of different rates (Lehnert et al., 2009).

The efficiency of fermentation in the brewing process, and the character and quality of the final product are linked to the amount and health quality of the yeast being pitched. Levels of organic acids, esters, higher alcohols, aldehydes and diacetyl can be influenced by the physiological conditions of the pitching yeast throughout fermentation and maturation, and consequently contribute to the overall organoleptic properties of the end product (Heggart et al., 2000). Industrial-scale systems utilizing immobilized yeast cells have been used for the production of low-alcohol beers (Willaert et al., 2006). The yeast metabolism during low-alcohol beer production is affected by environmental conditions and wort composition. This feature enables the brewer to optimize the flavour profile of the final product by interfering with yeast metabolism. The flow rate of O_2 and wort composition are used to control flavour compound concentration, which are modified according to the increase in biomass and the degree of fermentation (van Iersel et al., 1999).

The main problem arising from these methodologies is that low-alcohol beers suffer from having less body, low aromatic profile or sweet and worty off-flavours (Perpète and Collin, 1999; Montanari et al 2009; Sohrabvandi et al., 2010; Brányik et al., 2012). The sensorial quality of the final brew is very different to the original one; however low-alcohol beers are expected to be successful if their aroma profiles were as close as possible to the original brew (Catarino et al., 2009). It is for these reasons that low-alcohol beer production requires increased technological and economic concerns (Sohrabvandi et al., 2010). Electronic noses and electronic tongues have made great progress in their development, and the prediction of bitterness and alcoholic strength in beer by using an electronic tongue has recently been studied by our group (Arrieta et al 2010)

The aim of this present review is to evaluate the different flavour compounds in beer, focusing on those organoleptically undesirable compounds in low-alcohol beers. In addition, analytical methods currently used to detect flavour compounds in beer are also shown. Finally, techniques developed recently to solve these organoleptic problems are reported.

COMPONENTS OF AROMA AND FLAVOUR IN BEER

Beer flavour is the result of a complex interaction between hundreds of chemical compounds and their perception on taste and olfactory receptors (Saison D. et al., 2008). Consumer perception of low-alcohol beer quality is usually based on a complex mixture of expectations, which are associated with different effects of some sensory attributes such as colour, foam, flavour and aroma, mouthfeel and aftertaste (Ghasemi-Varnamkhasti et al., 2012). Through the tongue, compounds that impart taste can be sensed directly. Aroma will refer to any volatile compound arising out of the beverage that can be perceived on the nose or retro-nasally on the back of the mouth.

Table 1 shows the different taste compounds in beer and the organoleptic threshold of each component. The organoleptic threshold provides information on its impact on taste, aroma and flavour, but to consider these attributes of beer as the sum of the contributions of each individual compound is wrong because the interactions between components can affect the perception of them as a whole.

Figure 1 shows a simplified metabolic scheme of the formation of the main groups of flavour-active compounds by brewing yeast during beer fermentation.

In the next section, main components associated to flavour are revised.

Alcohols and phenols

During the aerobic growth of *S. cerevisiae*, both sugars and ethanol can be used as carbon and energy sources. Sugars can be metabolized via two different energy-producing pathways, oxidation or fermentation, the predominance of each one being dependent on the sugar concentration in the medium. The fermentative metabolism of glucose occurs when the glucose concentration is high enough, then ethanol and other alcohols are produced in this way (Blanco et al., 2008). Ethanol is an enhancer of some flavours such as those that lead to a sweet taste; and it is also a precursor of flavour-active esters. Furthermore, ethanol is also known to have a key role in the formation of the characteristic background flavour of beer, apart from giving a warming sensation to the mouth and stomach. In low-alcohol beers, a partial loss of flavour is inevitable as ethanol is removed by using different methods of dealcoholization. Therefore, low-alcohol beers lack the flavour components produced via fermentation in an appropriate concentration and balance (harmony) (Sohrabvandi et al., 2010; Caluwaerts, 1995). During primary beer fermentation, the major fraction of the volatile compounds are constituted by several higher alcohols, other than ethanol (Brányik et al., 2008), which are produced by yeast cells as by-products (Willaert et al., 2006). The final concentration of higher alcohols is determined by the efficiency of the corresponding amino acid uptake and sugar utilization rate (Brányik et al., 2008). Higher alcohols can be classified into aliphatic and aromatic alcohols.

The main aliphatic alcohols are n-propanol, isobutanol, 2-methylbutanol (amylalcohol) and 3-methyl butanol (isoamyl alcohol), and the main aromatic alcohols are 2-phenylethanol, tyrosol and tryptophol (Willaert et al., 2006).

Higher alcohols are synthesized by yeast during fermentation via the catabolic and anabolic pathways (amino acid metabolism) (Willaert et al., 2006). The immediate precursors are 2-oxo acids. Along the anabolic route, the 2-oxo acids derive from carbohydrate metabolism. Along the catabolic route (Ehrlich), the 2-oxo acids are formed through transamination of an amino acid. These are decarboxylated to form aldehydes, which are subsequently reduced to form the corresponding alcohols (Hazelwood et al., 2008). Wort composition and yeast strain fermentation conditions significantly influence the combination and levels of the higher alcohols that are formed (Willaert et al., 2006). The contribution of each biosynthetic pathway becomes in turn influenced by wort amino acid composition, the fermentation stage and yeast strain (Eden et al., 2001). For n-propanol the anabolic route is the only one possible contributing to its formation since there is no corresponding amino acid (Boulton and Quain, 2001).

High levels of nutrients (amino acids, oxygen, lipids, zinc) or increased temperature and agitation are conditions that promote yeast cell growth and stimulate the production of higher alcohols. Conversely, conditions which impose constraints to yeast growth, such as low temperature and high CO_2 pressure, decrease higher alcohol production to some extent (Willaert et al., 2006). García (1994) and Hough (1981) describe the level of oxygen, pH and temperature as the main parameters that influence higher alcohol production. While higher alcohol concentrations impart off-flavours, low concentrations make an essential contribution to the flavours and aromas (Hazelwood et al., 2008) hence, by changing these fermentation parameters, different higher alcohols related to flavours can be obtained in beer. Some of the characteristic flavours provided by higher alcohols in beer are:

a) Aliphatic higher alcohols contribute to the 'alcoholic' or 'solvent' aroma of beer and produce a warm mouthfeel (Willaert et al., 2006), the most significant contribution is owed to n-propanol, iso-butanol and isoamyl alcohols (2-methyl and 3-methyl butanol) (Brányik et al., 2008). N-propanol and 2-methylpropanol may cause 'rough' flavours and harshness of beer, amyl alcohols (2- and 3-methylbutanol) cause 'fruity' flavours (Šmogrovičová and Dömény, 1999). Isobutyl alcohol has an undesirable effect on beer quality when its concentration exceeds 20% of the total concentration of three alcohols: n-propanol, isobutanol, and amyl alcohol (Kobayashi et al., 2008).

b) The aromatic alcohol 2-phenylethanol causes 'sweet' or 'rose' flavours in beer (Šmogrovičová and Dömény, 1999), and makes a positive contribution to the beer aroma, whereas the aromas produced by tyrosol and tryptophol are undesirable (Willaert et al., 2006). Some monophenols present an unpleasant phenolic-like flavour, while others provide pleasant vanilla-like and smokey flavours. Vanillin was included in the reference standards for the beer flavour terminology system at a later stage (Sterckx et al., 2011).

Recently, it was shown that 4-vinylguaiacol contributes to the overall flavour of certain beer styles with a clove-like aroma (Vanbeneden et al., 2008), whereas 4-vinylsyringol may play a role in aged beer flavour (Callemien et al., 2006).

ACCEPTED MANUSCRIPT

Esters

The synthesis of aroma-active esters during beer brewing is of great importance because they represent a large group of flavour active compounds that confer a fruity-flowery aroma (Lehnert et al., 2008; Brányik et al., 2008; Šmogrovičová and Dömény, 1999). Esters can have very low flavour thresholds and a major impact on the overall flavour. The major esters can be subdivided into acetate esters and medium-chain fatty acid ethyl esters (Willaert et al., 2006).

The first group comprises acetate esters such as ethyl acetate (fruity, solvent-like), isoamyl acetate (banana) and phenylethyl acetate (roses, honey, apple). Ethyl acetate represents approximately one third of all esters in beers (Šmogrovičová and Dömény, 1999).

The second group of esters includes, among others, ethyl caproate and ethyl caprylate (both apple-like) (Brányik et al., 2008; Lehnert et al., 2008; Verstrepen et al., 2003).

Ester production by alcohol-acid reaction takes place in yeast fermentation as a CoA mediated reaction, both types of compounds being products of yeast metabolism (Garcia et al., 1994; Brányik et al., 2008). Two factors are of fundamental importance for the rate of ester formation: the availability of the two substrates (acetyl/acyl-CoA and alcohols) and the activity of enzymes (mostly alcohol acyltransferases) involved in the formation of esters. Consequently, the control of ester formation is difficult because many factors are involved in the regulation of enzyme activity or substrate availability (Lehnert et al., 2008). There are some additional factors that have an influence on ester production. These are temperature, CO_2 concentration or its pressure inside the fermenter, the presence of oxygen in the wort, pH and amino acid

 ACCEPTED MANUSCRIPT

concentration (Garcia et al., 1994). A thoughtful adaptation of these parameters allows brewers to steer ester concentrations and thus to control the fruity character of their beers (Verstrepen et al., 2003).

The relationship between total higher alcohols and total ester concentrations is an important indicator in evaluating beer flavour. Table 2 shows the relationship among aminoacids, their related higher alcohols and esters. It indicates whether the beer presents a more alcoholic or fruity character (Catarino et al., 2009). The overall flavour of beer depends on the relative contents of these compounds. The optimum higher alcohols-to-esters ratio for lagers is 4:1 to 4.7:1 (Šmogrovičová and Dömény, 1999). The presence of different esters can have a synergistic effect on the individual flavours, which means that esters can also have a positive effect on beer flavour, below their individual threshold concentrations. Volatile esters are common trace compounds in beer but are extremely important for flavour profile: they are desirable at low concentrations but undesirable at high concentrations (Verstrepen et al.,2003; Zhu et al., 2010). Moreover, the fact that most esters are present at concentrations around the threshold value implies that minor changes in concentration may have dramatic effects on beer flavour (Sterckx et al., 2011; Petersen et al., 2004). This problem has become very clear with the introduction of modern brewing practices (Verstrepen et al., 2003).

Carbonyl compounds

Carbonyl compounds can originate from raw materials, alcoholic fermentation or from a wide range of chemical reactions such as lipid oxidation, Maillard reaction, Strecker degradation and aldol condensation. Despite their concentrations being generally very low in beer, these compounds make an important and mostly unwanted contribution to flavour profile because of their low flavour thresholds. Moreover, the quantification of some carbonyl compounds can be used for the evaluation of a complete and proper fermentation. As a result, the quantitative determination of the volatile carbonyl content is very important (Saison et al., 2009). The most important carbonyl compounds involved in the aroma and taste profile of beer are vicinal diketones and aldehydes:

Ketones: the concentrations of two vicinal diketones (VDK), 2,3-butanedione (diacetyl) and 2,3-pentanedione, of which diacetyl is more flavour-active, are of critical importance for beer flavour (Brányik et al., 2008). Vicinal diketones are produced as by-products of the synthesis pathway of some amino acids during fermentation (Willaert et al., 2006). Diacetyl and 2,3-pentanedione results from the chemical oxidative decarboxylation of excess α-acetolactate and α-acetohydroxybutyrate, which are leaked to the extracellular environment from the valine biosynthetic pathway. The rate of vicinal diketones formation is limited by such chemical conversions. Acetoin and 2,3-butanediol are formed by yeast through a reductive reaction after diacetyl is reassimilated at the end of the main fermentation and maturation phases. Both compounds have relative high flavour thresholds. It seems that various enzymatic systems of the brewing yeast are involved in the reduction of vicinal diketones (Bamforth and Kanauchi, 2004; Van Bergen et al., 2005). Diacetyl is sensorily more important than 2,3-pentanedione (Willaert et al., 2006). It has a strong “butterscotch” aroma in concentrations above the flavour threshold, which is 0.10-0.15 ppm for lager beers (Brányik et al., 2008), it being approximately 10 times lower than that of pentanedione (Willaert et al., 2006). Diacetyl and 2,3-pentanedione have characteristic aromas and tastes described as ‘buttery’, ‘honey’ or ‘toffee-like’. At levels above 1 ppm it becomes increasingly ‘cheese-like’ and sharp (Šmogrovičová and Dömény, 1999).

Aldehydes: aldehydes arise in beer mainly during wort production (mashing, boiling). They are partially formed during fermentation from the yeast oxo-acid pool via the anabolic process and from exogenous amino acids via the catabolic pathway (Brányik et al., 2008). In typical lager beers, ethanol significantly increases aldehyde retention, leading to lower perception of the worty character. In alcohol-free beers, both the absence of ethanol and the higher level of mono and disaccharides such as maltose intensify such undesirable flavours (Perpète and Collin, 2000).

Acetaldehyde is the predominant carbonyl compound present in beer, representing approximately 60% of the total aldehydes (Guido et al., 2008). Its level varies during fermentation and ageing and usually lies within the range 2–20 mg/L (Šmogrovičová and Dömény, 1999). In alcohol-free beers 3-methylthiopropionaldehyde seems to be the key compound responsible for the worty off-flavour. The difficulty of extracting this compound by the usual headspace technique can explain why previous works have not provided evidence of it. At present, it seems that the organoleptic properties of alcohol-free beers are bonded to the synergic interaction of 3- and 2-methylbutanal to sulphur containing degradation products stemming from methional. Indeed, differences between alcohol-free and regular beers could arise from the solubilization of such compounds by ethanol (Perpète and Collin, 2000). Aldehydes have flavour threshold concentrations significantly lower than their corresponding alcohols. Almost without exception they have unpleasant flavours and aromas described as ‘grassy’, 'fruity', 'green leaves' and 'cardboard', depending on the real compound (Boulton and Quain, 2001).

Organic and fatty acids

The presence of 110 organic and short-chain fatty acids has been reported in beer (Boulton and Quain, 2001). A large portion of the total organic acids (ca. 50%) is derived from the wort, while the rest is produced or transformed as a result of yeast metabolism (Yamauchi et al., 1995). The majority of organic acids are derived directly from pyruvate, but there are organic acids with a short carbon skeleton which derive both from the incomplete turnover of the tricarboxylic acid cycle that occurs during anaerobic growth of yeast (Brányik et al., 2008; Boulton and Quain, 2001; Wales et al., 1980). Short-chain fatty acids (pyruvic, acetic, lactic, citric, succinic, malic,) impart a bitter flavour to beer. Long-chain fatty acids are primarily originated from wort and are undesirable for the taste of beer and foam stability (Brányik et al., 2008). Medium-chain fatty acids (caproic, caprylic and capric acid) afford off-flavours, characterized as rancid goaty flavour often called "caprylic" flavour (Boulton and Quain, 2001; Šmogrovičová and Dömény, 1999). This undesirable flavour normally arises from an excess of acid formation during fermentation or maturation. Their production is influenced mainly by the yeast strain used, wort composition, aeration and temperature. During maturation, the duration of the process, temperature used, and physiological state of yeasts are critical factors that determine yeast autolysis and concurrent release of fatty acids. Analyzing this group of compounds is recognized as a valuable method to monitor the maturation progress (Horák et al., 2008).

In general, organic acids have sour flavours and contribute to the lowering of pH that occurs during fermentation (Boulton and Quain, 2001). In addition to sourness, individual organic acids are reported to have characteristic flavours, which are dependent on the production method and conditions. For example, succinic is described as having a salty or bitter taste (Whiting, 1976). Short chain fatty acids are usually present in beer at total concentrations of 20–150 ppm. Butyric and iso-butyric acids may cause a 'butyric' or 'rancid' flavour at a concentration above 6 ppm; valeric and iso-valeric acids cause 'old hop' and 'cheesy' flavours (Šmogrovičová and Dömény, 1999). Usual contents of organic acids in regular beers are 100-200 ppm for pyruvic, 10-50 ppm for acetic, 50-300 ppm for lactic, 100-150 ppm for citric, 50-150 ppm for succinic and 30-50 ppm for malic (Boulton and Quain, 2001; Coote and Kirsop, 1974; Klopper et al., 1986). The total of fatty acids in regular beers (caprylic, caproic and capric acids) represent about 75-80% (Boulton and Quain, 2001) and their concentration thresholds are approximately 5 ppm for caproic acid and 10 ppm for caprylic and capric acids. Lauric acid may cause 'soapy' flavors at a concentration higher than 6 ppm (Šmogrovičová and Dömény, 1999). The strategy for the control of the production of these acids is based on the regulation of yeast growth (Yamauchi et al., 1995; Brányik et al., 2008).

FLAVOUR DEFECTS IN ALCOHOL-FREE BEER

When producing low-alcohol beer, it is important to maintain the natural flavour of a regular beer. Unfortunately, the taste of the final product is not currently as good as that of regular alcoholic beer (Sohrabvandi et al., 2010). Taste defects in low-alcohol beer are due to an undesirable effect derived from the main ways of eliminating or reducing the ethanol in beer.

These processes are responsible for the characteristic sensorial defects in the final product. Thus, beer in which alcohol production has been prevented or reduced at an early stage of fermentation is dull and inharmonious in taste and has an immature flavour. The fermentation activity can be prevented quickly by rapid cooling to 0ºC, pasteurization and/or by the removal of yeast from fermenting wort (Brányik et al., 2012). Its flavour profile is characterized by worty off-flavours and a lack of the pleasant fruity (estery) aroma found in regular beers (Sohrabvandi et al., 2010; Perpète and Collin, 1999) due to insufficient wort aldehyde reduction and a lack of fusel alcohols and ester production (Lehnert et al., 2009). Besides, beer dealcoholized by ethanol removal is characterized by a loss of volatiles (higher alcohols, esters) accompanying ethanol removal (Lehnert et al., 2009). Thus, when using thermal processes low-alcohol beer suffers heat damage and aroma and flavour compounds, more volatile than ethanol, are evaporated. The vacuum distillation process consists of two stages: evaporation under high vacuum followed by cold condensation. Both thin film evaporators and atomizing evaporators with vacuum chamber have been used, as well as the combination of both methods. In this case, flavour compounds should be restored after dealcoholization (Sohrabvandi et al., 2010). Using an aroma recovery unit, 6% and 20% of the originally present higher alcohols and esters, are respectively returned (Brányik et al., 2012). Low-alcohol beers produced by a membrane process have less body and a low aromatic profile. The membrane process can be divided into dialysis and reverse osmosis. Dialysis operates at a low temperature and uses the selectivity of a semi-permeable membrane. Certain molecules pass through the membrane into the dialysis medium, depending on the pore size and surface properties of the membrane (Sohrabvandi et al., 2010; Brányik et al., 2012). In this case, other components of beer besides ethanol, such as higher alcohols and esters, are almost completely removed (Brányik et al., 2012). In the reverse osmosis process, beer is passed through a semi-permeable membrane under high pressure conditions (Sohrabvandi et al., 2010). In this case, besides the losses of volatiles, other large molecules such as aroma and flavour compounds are removed (Brányik et al., 2012). .

Ethanol contributes directly to the flavour of beer, giving rise to a warming character and flavour perception of other beer components (Huges et al., 2001). Ethanol increases aldehyde retention, leading to a lower perception of the worty taste. In regular beers the retention of aldehydes is 32-39% as opposed to 8-12% retention in alcohol-free beers (Brányik et al., 2012).

Some aldehydes present in wort have high flavour potency (3-methylbutanal, 2-methylbutanal, hexanal, heptanal, etc.) (Brányik et al., 2008). Acetaldehyde causes 'green vegetation' or 'vegetable' flavour at concentrations of 20–25 ppm (Šmogrovičová and Dömény, 1999).

Wort carbonyls contribute largely to the unpleasant worty taste detected particularly in low-alcohol beer produced by limited fermentation. The yeast metabolism reduces these substances to less flavour active ones (Lehnert et al., 2009; Brányik et al., 2008). During batch fermentations aldehyde reduction is relatively rapid, but it may not be sufficient at the speed of the limited fermentation in continuous systems (Lehnert et al., 2008). In fact, a good compromise was reached between alcohol formation and carbonyl reduction by optimizing the residence time and temperature of the continuous low-alcohol beer production process (Lehnert et al., 2008; Brányik et al., 2008).

Whole fatty acids are undesirable components of beers in two ways. First of all from the point of view of taste and the secondly due to their potential to adversely affect foam

 ACCEPTED MANUSCRIPT

performance (Boulton and Quain, 2001). Furthermore, the pH value and taste of beer are greatly influenced by its organic/inorganic acid content (Zhu et al., 2010; Haddad et al., 2008).

The most significant impact of low-alcohol beer produced by removing ethanol is that part of the volatile fraction, such as higher alcohols and esters, both good flavour components of beer, disappears. All dealcoholization technologies lead to significant losses of volatiles, although minimal losses occur in the case of the membrane process. These flavour imperfections increased the need to correct them, for example with additives (Brányik et al., 2012).

The colour of beer is also affected by the dealcoholization processes. The thermal process tends to highten the colour, while membrane processes decrease the colour of low-alcohol beers. Whatever the dealcoholization method used, bitterness and foam stability are usually impaired (Brányik et al., 2012) and beers are more prone to microbial contamination due to the low ethanol content as well as the presence of fermentable sugars. This feature has to do with the positive synergistic effect of ethanol during the pasteurization of beer. Thus, since low-alcohol beers need higher pasteurization temperatures, an adverse influence on flavour characteristics and colloidal stability of the beer is caused. Indeed, when low-alcohol beers are produced by restricted fermentation procedures, beers with high fermentable sugar content are obtained and, hence, they are prone to be contaminated more easily (Sohrabvandi et al., 2010). The diacetyl/pentanedione ratio can reflect the relationship between flavours and microbes in beer. The diacetyl/pentanedione ratio was found to be approximately 1 when microorganisms were not detected, but polluted beer was found to have a higher ratio. Pentanedione was reduced significantly once the beer was highly contaminated by microbes during fermentation, whereas a prominent increase of diacetyl was recorded concurrently. When the concentration of diacetyl in beer exceeded the endurable threshold, the consumers were able to detect the presence of diacetyl when tasting (Tian, 2010).

Furthermore, it has been pointed out that contamination with spoilage microorganisms might result in off-flavours such as rotten eggs, cooked cabbage, celery-like flavour, vinegary flavour, phenolic flavour, lactic acid, diacetyl and acetaldehyde (Sohrabvandi et al., 2010).

POSSIBLE SOLUTION STRATEGIES

If ethanol productivity were the only quality criterion, it would be relatively easy to control and optimize the brewing process. However, during beer production, the well-balanced aroma and flavour of the final product are equally or even more important than efficient fermentation and high ethanol yield. Presently, different strategies to solve this problem are being investigated because of the great economic importance for breweries.

a) Control strategies based on the manipulation of parameters during fermentation.

Van Iersel et al. (1999) research reveals that anaerobic conditions inhibit microorganism growth and stimulate ester production, whereas oxygen stimulates growth but may cause oxidative off-flavours. By increasing the temperature, yeast metabolism and ester production will increase. By the introduction of regular aerobic intervals, an optimum can be reached between the supply of oxygen for yeast growth and the prevention of oxidation of the low-alcohol beer (Willaert et al., 2006; Lenhert et al., 2009). By changing the mashing process, it is possible to modulate the profile of wort sugar to obtain a limited fermentability and hence, a low alcohol content. This can be achieved, for example, with a high mashing temperature (75-80ºC) causing a ß-amilase inactivation. The flavour of these beers is good; however, some worty flavours have been reported (Brányik et al., 2012). Nowadays, temperature, feed volume, wort gravity, wort composition, residence time, and aeration are the main parameters considered for optimisation in order to find a constant and optimum well-balanced taste in low-alcohol beer (Willaert et al., 2006; Lenhert et al., 2009).

b) *Use of special yeast strains that form less ethanol during complete fermentation of wort sugars.*

The reduction of ethanol production could be achieved by metabolic engineering of the carbon flux in yeast resulting in an increased formation of other fermentation products such as glycerol. However, only by-products that do not disturb the taste of beer are acceptable. Nevoigt et al. (2002) explains that the GPD1 gene encoding the glycerol-3-phosphate dehydrogenase was overexpressed in an industrial lager brewing yeast (*Saccharomyces cerevisiae ssp. Carlsbergensis*) to reduce the content of ethanol in beer. The amount of glycerol was increased 5.6 times and ethanol was decreased by 18% when compared to the wild-type. Overexpression of GPD1 does not affect the consumption of wort sugars. Minor changes in the concentration of higher alcohols, esters and fatty acids could only be observed in beer produced by GPD1. However, the concentrations of several other by-products, particularly acetoin, diacetyl and acetaldehyde, were considerably increased.

Other *Saccharomyces* strains have been studied in order to make low-alcohol beers. *Saccharomyces ludwigii* at low temperature and low density can be applied in controlled fermentation due to its inability to ferment maltose (the most abundant sugar in wort) and maltotriose. *Saccharomyces ludwigii* showed a higher volatile compounds formation (higher alcohol and esters), in spite of remaining off-flavours (aldehyde and diacetyl) (Mohammadi et al., 2011; Brányik et al., 2012).

In controlled fermentation it is important to perform a selection of yeast strain as well as the operation conditions used in each dealcoholization process. All the factors involved will determine the sensory quality of the final alcohol-free beer.

c) *Emerging technologies to produce non-alcoholic beers by removing ethanol from a completely fermented beer.*

Some technologies have been developed as a complement to thermal dealcoholization to decrease the thermal damage and loss of volatiles. Aroma recovery systems allow the beer to be rectified with the aroma compounds, which can be commercial or elaborated from processed beer (Lipnizki et al. 2002). Nowadays, many of them are based on the recovery of natural aroma compounds from beer (Catarino and Mendes, 2011)

Pervaporation is a newly developed process that considers the extraction of aromas from multicomponent mixtures. Thus, She and Hwang (2006) analyzed the effect of pervaporation operating conditions (concentration and temperature) and the membrane properties on the separation of multicomponent mixtures representing real flavour systems. On the other hand, they reported the recovery of key flavour compounds (alcohols, esters and aldehydes) from real solutions (apple essences, orange aroma and black tea distillate), by using different membranes. Catarino et al. 2009 developed a process to extract aromas from the original beer by using a

POMS (polyoctylmethylsiloxane) membrane. Seven aroma compounds were selected to characterize the beer profile, four alcohols (ethanol, propanol, isobutanol, and isoamyl alcohol), two esters (ethyl acetate and isoamyl acetate) and one aldehyde (acetaldehyde). This beer aroma is intended to correct the aroma profile of the same beer after a dealcoholization process. The results show that pervaporation is an effective process for recovering aroma compounds from beer.

An industrial process by using *spinning cone column distillation* for producing non-alcoholic beer (ethanol < 0.5 vol%) with improved flavour profile has been recently investigated by Catarino and Mendes (2011). This process is a variation of vacuum distillation, which uses a column with a special design, the spinning cone column (SCC). SCC consists of a gas-liquid countercurrent device where the stripping medium (e.g. water vapour) extracts the ethanol from the beverage. The dealcoholized beer is blended with fresh alcoholic beer and natural extracted aroma compounds. These aroma compounds are obtained by pervaporation of the original beer, using polyoctylmethylsiloxane/polyetherimide (POMS/PEI) membranes. The main advantages of SCC distillation comprise low residence time, high contact area between liquid and vapour, low pressure in the column and moderate temperatures, which minimizes the thermal impact on beer.

However, most of these strategies involve difficulties due to the control exerted by the laws of some countries in relation to the alcoholic phase separated during the processes of dealcoholization (ej: distillation process).

CONCLUSIONS

In recent years, there has been an increased market share for low-alcohol beers. This is mainly due to health and safety reasons and increasingly strict social regulations. Low-alcohol beer consumers seek a product as close as possible to normal beer, but the dealcoholization features give these kinds of beers an artificial and immature taste. When ethanol is removed from regular beer, there are basically four consequences for low-alcohol beers:

- In incompleted fermentation, carbonyl compounds are reduced only slightly, therefore confering unpleasant flavours.

- A lack of flavour due to the elimination of both ethanol and other alcohols during the dealcoholization process.

- Some favourable compounds are missing because ethanol operates as a solvent.

- Low-alcohol beer contamination with spoilage microorganisms increase due to the lack of ethanol.

For these reasons, low-alcohol beers have given rise to social, technological, and economical interests, which will require a comprehensive analysis of these flavour compounds.

In this review, we have shown the flavour compounds of beer, in order to determine those associated with sensorial defects of taste in low-alcohol beer.

Acknowledgements:

CSIC collaboration in the analysis with the mass spectrometer (UPLC-QToF-MS) is gratefully acknowledged.

Financial support from Junta de Castilla y León (VA332A12-2) is gratefully acknowledged.

 ACCEPTED MANUSCRIPT

REFERENCES

Arrieta, A. A., Rodríguez-Méndez, M. L., de Saja, J. A., Blanco, C. A. and Nimubona, D. (2010). Prediction of bitterness and alcoholic strength in beer using an electronic tongue. *Food Chemistry*, **123**: 642-646.

Bamforth, C.W. and Kanauchi, M. (2004). Enzymology of vicinal diketone reduction in brewer´s yeast. *Journal of the Institute of Brewing*. **110:** 83-93.

Belisario-Sánchez, Y.Y., Taboada-Rodríguez, A., Marín-Iniesta, F. and López-Gómez, A. (2009). Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1, 1-diphenyl-2-picrylhydrazyl method. *Journal of Agricultural and Food Chemistry*. **57 (15):** 6770-6778.

Blanco, C.A. and Rayo, J. (2008). Improving industrial full-scale production of baker´s yeast by optimizing aeration control. *Journal of AOAC International*, **91 (3):** 607-613.

Boulton, C. and Quain, D. (2001). The biochemistry of fermentation. **In:** Brewing yeast and fermentation, pp 69-142. Eds., Blackwell Science, Oxford, U.K.

Brányik, T. Silva, D.P., Baszczyn□ski, M., Lehnert, R. and Almeida e Silva, J.B. (2012). A review of methods of low alcohol and alcohol-free beer production. *Journal of Food Engineering*. **108:** 493-506.

Brányik, T., Vicente, A.A., Dostálek,P. and Teixeira, J.A. (2008). A review of flavor formation in continuous beer fermentations. *The Institute of Brewing & Distilling*, **114 (1):** 3-13.

 ACCEPTED MANUSCRIPT

Callemien, D., Dasnoy, S. and Collin, S. (2006). Identification of a stale-beer-like odorant in extracts of naturally aged beer. *Journal of Agricultural and Food Chemistry*, **54(4):** 1409–1413.

Caluwaerts, H.J.J. (1995). Process for the manufacture of an alcohol-free beer having the organoleptic properties of a lager type pale beer. U.S. Patent 5, 384,135.

Catarino, M., Mendes, A., Madeira, L.M. and Ferreira, A. (2007). Alcohol removal from beer by reverse osmosis. *Separation Science and Technology*, **42 (13):** 3011–3027.

Catarino, M., Ferreira, A. and Mendes, A. (2009). Study and optimization of aroma recovery from beer by pervaporation. *Journal of Membrane Science*, **341:** 51–59.

Catarino, M. and Mendes, A. (2011). Non-alcoholic beer—A new industrial process. *Separation and Purification Technology*, **79:** 342– 351.

Coote, N. and Kirsop, B.H. (1974). The content of some organic acids in beer and other fermented media. *Journal of the Institute of brewing*, **80:** 474-483.

Eden, A., Van Nedervelde, L., Drukker, M., Benvenisty, N. and Debourg, A. (2001). Involvement of branched-chain amino acid aminotransferases in the production of fusel alcohols during fermentation in yeast. *Applied Microbiology and Biotechnology*, **55:** 296-300.

Hazelwood, L.A., Daran, J-M., van Maris, A.J.A, Pronk, J.T. and Dickinson, J.R. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology*, **74:** 2259-2266.

Heggart, H., Margaritis, A., Stewart, R., Pikington, H., Sobczak, J. and Russell, I. (2000). Measurement of brewing yeast viability and vitality: a review of methods. *Technical Quarterly – Master Brewers Association of America*, **37(4):** 409–430.

Klopper, W. J., Angelino, S.A.G.F., Tuning, B. and Vermeire, H.A. (1986). Organic acids and glycerol in beer. *Journal of the Institute of Brew*, **92:** 225-228.

Kobayashi, M., Shimizu, H. and Shioya, S. (2008) Beer volatile compounds and their application to low-malt beer fermentation. *Journal of Bioscience and Bioengineering*, **106 (4):** 317–323.

García, A.A., Cancho Grande, B. and Gandara, J.S. (2004). Development of rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for the determination of polyphenols in alcohol-free beers. *Journal of Chromatography*, **1054:** 175–180.

García, A.I., García, L.A. and Díaz, M. (1994). Fusel alcohols production in beer fermentation. *Process Biochemistry*, **29:** 303-309.

Ghasemi-Varnamkhasti, M., Mohtasebi, S.S., Rodriguez-Mendez, M.L., Lozano, J., Razavi, S.H., Ahmadi, H. and Apetrei, C. (2012). Classification of non-alcoholic beer based on aftertaste sensory evaluation by chemometric tools. *Expert Systems with Applications*, **39:** 4315-4327.

Guido, L., Rajendram, R. and Barros, A.A. (2008). Pitching yeast and beer flavour: **In:** Beer in health and disease prevention. Preedy, V.R., Ed., Academic Press, USA.

Haddad, R., Sparrapan, R., Kotiaho, T. and Eberlin, M.N. (2008). Easy Ambient Sonic-Spray Ionization-Membrane Interface Mass Spectrometry for Direct Analysis of Solution Constituents. *Analytical Chemistry*, **80:** 898–903.

Horák, T., C□ulík, J., Jurková, M., C□ejka, P. and Kellner, V. (2008). Determination of free medium-chain fatty acids in beer by stirbar sorptive extraction. *Journal of Chromatography A*, **1196–1197:** 96–99.

Hough, J.S., Briggs, D.E., Stevens, R. and Young, T.W. (1981). Metabolism of wort by yeast. **In:** Malting and Brewing science, vol II, Hopped wart and beer, pp 566-641. An Aspen Publication, Ed., Chapman and Hall, New York.

Labanda, J., Vichi, J., Llorens, J. and López-Tamames, E. (2009). Membrane separation tehcnology for the reduction of alcoholic degree of a white model wine. *LWT- Food Science and Technology*, **42(8):** 1390-1395.

Lehnert, R., Kuřec, M. and Brányik, T. (2008). Effect of oxygen supply on flavor formation during continuous alcohol-free beer production: A model study. *American Society of Brewing Chemisys*, **66(4):** 233-238.

Lehnert, R., Novák, P., Macieira, F., Kuřec, M., Teixeira, J.A. and Brányik, T. (2009). Optimisation of lab-scale continuous alcohol-free beer production. *Czech Journal Food Science*, **27:** 267–275.

Lipnizki, F., Olsson, J. and Trägårdh, G. (2002). Scale-up of pervaporation for the recovery of natural aroma compounds in the food industry. Part 1. Simulation and Performance. *Journal of Food Engineering*, **54:** 183–195.

Mohammadi, A., Razavi, S.H., Mousavi, S.M. and Rezaei, K. (2011). A comparision between sugarconsumption and ethanol production in wort by immobilized *Saccharomyces cerevisiae, Saccharomyces ludwigii, Saccharomyces rouxii* on brewer´s spent grain. *Brazilian Journal of Microbiology*, **42**: 605-615.

Montanari, L., Marconi, O., Mayer, H. and Fantozzi, P. (2009). Production of alcohol-free beer. **In:** Beer in Health and Disease Prevention, pp. 61–75. Preedy, V.R., Eds., Elsevier Inc., Burlington, MA.

Nevoigt, E., Pilger, R., Mast-Gerlach, E., Schmidt, U., Freihammer, S., Eschenbrenner, M., Garbe, L. and Stahl, U. (2002). Genetic engineering of brewing yeast to reduce the content of ethanol in beer. *FEMS Yeast Research*, **2:** 225-232.

Perpète P. and Collin S. (1999). Contribution of 3-methylthiopropionaldehyde to the worty flavor of alcoholfree beers. *Journal of Agricultural and Food Chemistry*, **47**: 2374–2378.

Perpète P. and Collin S., (2000), Infuence of beer ethanol content on the wort flavour perception. *Food Chemistry*, **71:** 379-385.

Petersen E. E., Margaritis A., Stewart R. J., Pilkington P. H. and Mensour N. A. (2004). The effects of wort valine concentration on the total diacetyl profile and levels late in batch fermentations with brewing yeast Saccharomyces carlsbergensis. *Journal of the American Society of* Brewing *Chemists*, **62:** 131-139.

Piddocke, M.P. (2009). The effect of high gravity on brewer's yeast metabolism- physiological studies and "-omics" analyses. PhD thesis. Technical University of Denmark, Department of Systems Biology.

Saison, D., De Schutter, D.P., Delvaux, F. and Delvaux, F.R. (2008). Optimisation of a complete method for the analysis of volatiles involved in the flavor stability of beer by solid-phase microextraction in combination with gas chromatography and mass spectrometry. *Journal of Chromatography A*, **1190:** 342-349.

Saison, D., De Schutter, D.P., Delvaux, F. and Delvaux, F.R. (2009). Determination of carbonyl compounds in beer by derivatisation and headspace solid-phase microextraction in combination with gas chromatography and mass spectrometry. *Journal of Chromatography A*, **1216:** 5061-5068.

She, M. and Hwang, S.T. (2006). Recovery of key components from real flavour concentrates by pervaporation. *Journal of Membrane Science*, **279:** 86-93.

Šmogrovičová, D. and Dömény, Z. (1999). Beer volatile by-product formation at different fermentation temperature using immobilised yeasts. *Process Biochemistry*, **34:** 785–794.

Sohrabvandi, S., Mousavi, S.M., Razavi, S.H., Mortazavian, A.M. and Rezaei, K. (2010). Alcohol-free beer: Methods of production, sensorial defects, and healthful effects. *Food Reviews International*, **26:** 335–352.

Sterckx F. L., Missiaen J., Saison D., Delvaux F. R. (2011). Contribution of monophenols to beer flavour based on flavour thresholds, interactions and recombination experiments. *Food Chemistry*, **126:** 1679-1685.

Tian, J. (2010). Determination of several flavours in beer with headspace sampling–gas chromatography. *Food Chemistry*, **123:** 1318–1321.

Vanbeneden, N., Gils, F., Delvaux, F. and Delvaux, F. R. (2008). Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts. *Food Chemistry*, **107(1):** 221–230.

Van Bergen, B., Sheppard, J.D. and Jardim, A. (2005). Purification, identification and characterisation of diacetil reductase enzymes in ale and lager brewing yeast. **In:** Proceedings of the European Brewing Convention Congress, Prague, pp 139/1-139/6. IRL Press, Eds., Oxford.

Van Iersel, M.F.M., van Dieren, B., Rombouts, F.M. and Abee, T. (1999). Flavor formation and cell physiology during the production of alcohol-free beer with immobilized Saccharomyces cerevisiae. *Enzyme and Microbial Technology*, **24:** 407-411.

Verbelen, P.J and Devaux, F.R. (2009). Brewing yeast in action: beer fermentation. **In:** Applied Mycology, pp 110-136. Rai, M and Bridge, P.D., Eds., CAB International, UK.

Verstrepen, K. J., Derdelinckx, G. and Delvaux, F. R. (2003). Esters in beer-part.1: The fermentation process: more than ethanol formation. *Cerevisia*, **28:** 41-49.

Verstrepen, K.J., Derdelinckx, G., Dufour, J.P., Winderickxm J., Thevelein, J.M., Pretorius, I.S. and Delvaux, F.R. (2003). Flavour-active esters: adding fruitiness to beer. *Journal of Bioscience and Bioenergy*, **96:** 110–118.

Wales, D.S., Cartledge, T.G. and Lloyd, D. (1980). Effects of glucoserepression and anaerobiosis on the activities and subcellular distribution of tricarboxylic acid cycle and associated enzymes in Saccharomyces carlsbergensis. *Journal of general microbiology*, **116:** 93-98.

Whiting, G.C. (1976) Organic acid metabolism by yeast during fermentation of alcoholic beverages: a review. *Journal of the Institute of Brewing*, **82:** 84-92.

Willaert, R. and Nedovic, V.A. (2006). Primary beer fermentation by immobilized yeast – a review on flavour formation and control strategies. *Journal of Chemical Technology and Biotechnology*, **81:** 1353–1367.

Zhu, L., Zhong Hu, Z., Gerardo Gamez, G., Wai Siang Law, W., HuanWen Chen, H., ShuiPing Yang, S., Konstantin Chingin, K., Roman, M., Balabin, R., Rui Wang, R., TingTing Zhang, T. and Renato Zenobi, R. (2010). Simultaneous sampling of volatile and non-volatile analytes in beer for fast fingerprinting by extractive electrospray ionization mass spectrometry. *Analysis of Bioanalytical Chemistry*, **398:** 405–413.

Table 1. Different taste compounds in beer and organoleptic threshold.

Compound	Taste in beer	Organoleptic threshold (ppm)	Reference
Higher Alcohols			
n-propanol	Alcohol	800,00	Kobayashi, 2008
isobutyl alcohol	Alcohol	200,00	Kobayashi, 2008
amyl alcohol	Alcohol, banana, medicinal, solvent, fruity	65,00	Kobayashi, 2008
isoamyl alcohol	Alcohol, banana, sweetish, aromatic	70,00	Kobayashi, 2008
2-phenyl etanol	Roses, sweetish, perfumed	125,00	Kobayashi, 2008
Esters			
ethyl acetate	Solvent, fruity, sweetish	21,00	Piddocke, 2009
isoamyl acetate	Banana, apple, solvent, estery, pear	1,40	Piddocke, 2009
2-phenylethyl acetate	Roses, honey, Apple, sweetish	3,80	Kobayashi, 2008
ethyl caproate	Sour apple, anniseed	0,17	Willaert, 2006
ethyl caprylate	Sour Apple	0,30	Willaert, 2006
Vicinal diketones			
diacetyl	Butter	0,15	Kobayashi, 2008
2,3-pentanedione	Honey, toffee-like	1,00	Willaert, 2006
Organic and fatty acids			
caprylic	Goaty, fatty acid	14,00	Verbelen and Devaux, 2009
caproic	Goaty, fatty acid	8,00	Verbelen and Devaux, 2009
capric	Waxy, rancid	10,00	Verbelen and Devaux, 2009
Aldehydes			
acetaldehyde	Grassy, green leaves, fruity	25,00	Kobayashi, 2008

Table 2. Formation sequence from amino acids to alcohols and esters.

Amino acids	Higher alcohols	Esters
Valine	isobutanol	isobutyl acetate
Leucine	3-metilbutanol (isoamyl alcohol)	isoamyl acetate
Isoleucine	2-methylbutanol (amyl alcohol)	amyl acetate
Phenylalanine	2-phenylethanol	phenyl ethyl acetate

Figure and table captions

Figure 1. Flavour active compounds in brewing yeast