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REGIONE AUTÒNOMA DE SARDIGNA
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FSE 2007-2013
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obiettivo competitività regionale e occupazione

UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Biotecnologie microbiche agroalimentari

Ciclo XXVII

Development, characterization and shelf life of craft wheat beer

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Anno accademico 2013- 2014

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RVRALIS ET SILVESTRIS SAPIENTIA

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La presente tesi è stata prodotta durante la frequenza del corso di dottorato in "Scienze e Biotecnologie dei Sistemi Agrari e Forestali e delle Produzioni Alimentari" dell'Università degli Studi di Sassari, a.a. 2013/2014 - XXVII ciclo, con il supporto di una borsa di studio finanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 "Finanziamento di corsi di dottorato finalizzati alla formazione di capitale umano altamente specializzato, in particolare per i settori dell'ICT, delle nanotecnologie e delle biotecnologie, dell'energia e dello sviluppo sostenibile, dell'agroalimentare e dei materiali tradizionali".

Ilaria Mascia gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 - Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.)

To my grandmother

LIST OF PAPERS

This PhD thesis is based on the following papers:

I. Preliminary characterization of an Italian craft durum wheat beer
Mascia, I., Fadda, C., Dostálek, P., Olšovská, J., and Del Caro, A. (2014), **Journal of the Institute of Brewing**, DOI 10.1002/jib.176

II. Novel starters for old processes: use of *Saccharomyces cerevisiae* strains isolated from artisanal sourdough for craft beer production at a brewery scale
Marongiu, A., Zara, G., Legras, J. L., Del Caro, A., Mascia, I., Fadda, C., and Budroni, M. (2014), **accepted for publication in Journal of Industrial Microbiology & Biotechnology**

III. Is it possible to create an innovative craft durum wheat beer with sourdough yeasts? A case study
Mascia, I., Fadda, C., Dostálek, P., Karabin, M., Zara,G., Budroni, M., and Del Caro, A.
Submitted to Journal of the Institute of Brewing, 2014

IV. Assessment of craft durum wheat beer during aging
Mascia, I., Fadda, C., Karabin, M., Dostálek, P., and Del Caro, A.
Submitted to Food Chemistry, 2014

Other works done during the thesis period are:

V. The world of microbreweries in Sardinia and development of a craft durum wheat beer
Mascia, I., INDC 2013, 13th International Nutrition & Diagnostics Conference, Olomouc (Czech Republic), 26-29 August 2013

VI. Is it possible to create an innovative beer with an old autochthonous yeast?
Mascia, I., Dostálek, P., Karabin, M., Fadda, C., and Del Caro, A., 11th International trends in brewing, Ghent (Belgium), 13-17 April 2014

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1 INTRODUCTION

1.1. Beer and its components

Beer is one of the most worldwide consumed beverages. It has been a popular drink for thousand years with records of its consumption dating back to the ancient cities of Mesopotamia (6000 years before Christ). Beer technological process was known in ancient Egypt (3000 years B.C.) and it was acquired also by Romans and Greeks. In the Mediterranean area, beer was gradually replaced by wine but its production became important in northern areas, particularly in central Europe. During the medieval times beer was consumed in large amounts as it was considered microbiologically safer than water; this was due to the anti-bacteriological properties of alcohol and to the boiling of water in the brewing process (Baxter *et al.*, 2001). Nowadays, beer is part of the cultural background of several European populations and it is the chosen drink to accompany food in different geographical areas (Bamforth, 2003a).

Beer is a complex beverage that is produced from different raw materials of agricultural origin. The main ones are barley, hops, water and yeast, whose characteristics influence the quality of beer. Knowledge of all ingredients is therefore essential to ensure an optimal process technology.

Barley is the principal raw material of beer. It is used in brewing after conversion into malt, a process aimed at reducing the grains' starch into sugars readily available for brewing.

The importance of barley is related to its working by a wort filtration layer in the phase of filtration. This is due to its high starch content and to the husk adherence to the grain that occurs even after the threshing process and the transformation of barley into malt.

In some countries, other cereals, such as maize, rice, sorghum, barley and unmalted wheat, are often used as additions. In Germany, for example, the use of wheat and other malted cereals is considered for producing top fermented beers.

The aromatic and bitter character of beer is due to the presence of hops. The high quality of hops is synonymous with an equally high-quality of beer (Kunze, 1999). Water represents the most important raw material of beer in quantitative terms. Every step of the brewing is influenced by the chemical compounds in water and by its biological composition. To overcome this issue, a water treatment is essential (Fergus *et al.*,2006).

The presence of yeast allows the development of the alcoholic fermentation. Since fermentation is dependent on the activity of the yeast, the production of beer depends on the type of the yeast. Fermentation produces ethanol and carbon dioxide as major metabolites but several other substances, such as esters, higher alcohols and acids, are formed as secondary metabolites. The secondary metabolites are responsible for the beer flavor and differences in their concentration result in a different character of beer flavor, making a beer unique to a drinker (Briggs *et al.*, 2004).

In a small part of worldwide beer production (close to 10%), barley malt is not the main raw material. In fact, other raw materials are often used to give a unique and distinctive taste to beer. An example is given by the weissbiers, in Germany, in which malted wheat is used, or the so-called kafir beers, in South Africa, in which the sorghum is used. However the barley malt is the key component in most beers and rarely is used for less than 50%. It often represents the sole source of fermentable carbohydrates. It can be said that a high-quality beer is synonymous with a high quality of malt (Bamforth, 2003b).

In Italian law, the term "beer" is reserved for a product obtained by alcoholic fermentation of a wort made either with malted barley or wheat (or a mixture) and water, using strains of *Saccharomyces carlsbergensis* or *Saccharomyces cerevisiae*. Adding hops or its derivatives gives beer its characteristic bitter taste. Barley and wheat malt may be substituted with other grains or sugary and starchy raw materials, to a maximum of 40% (w/w) calculated on the dried wort extract (Italian Law n. 1354/62, 1998).

In 2012, Italian beer production was ~13.5 million hL, representing about 75% of Italian consumption (17.6 million hL, corresponding to 29.5 litres per capita). Beer consumption in Italy is at the bottom of the European ranking scale with an average that is less than 40% of the European average, and three to five times lower than that of countries with the highest consumption (Czech Republic with 144 litres per capita, Austria with 108 litres per capita and Germany with 105 litres per capita) (ASSOBIRRA, 2014).

1.2. The process of beer production

The technological process for brewing is composed of several steps. Each step has a relevant importance in beer production. The first step regards the selection of barley: the malting barley should have a moisture content lower than 12% and a relatively low total N content with high viability and with endosperm of mealy texture that hydrates readily and possesses cell walls that are readily degraded. Once selected, the barley is stored to possibly allow barley to free up of innate dormancy. The malting process of barley begins with the steeping of the grains in water, in order to stimulate the germination of the seeds. During this phase the seed absorbs moisture and humidity from initial values of 12–14% to about 43–46%. The process consists of a series of repeated cycles of immersion in water at a temperature of 14–18°C alternated with times when the grains are taken off the water in order to provide them oxygen. When the moisture of the seed is close to 30% the embryo gives boot to germination by synthesizing growth hormones (notably gibberellins) that are sent to the aleurone. This one becomes receptive to the hormone action that will trigger enzyme synthesis. The produced enzymes catalyze the breakdown of the endosperm starch, of proteins and glucans ("modification"). After the steeping process, the malting proceeds with the germination, in which the degradation of the cell walls of endosperm and of most of the proteins takes place. This process occurs in 3–6 days at a temperature of 16–20°C. When migrating from the proximal to the distant end of the seed, the enzymes synthesized by the aleurone digest the cell walls (β-glucanase family of enzymes, pentosanases) and the proteins (proteinase family). This process causes the softening of the grain. At the end of this phase the barley is called green malt and contains a high quantity of enzymes that will be essential for seed starch degradation into soluble sugars that are then fermented by the yeast. The last step of the malting is the kilning: it consists in the drying of the grain in order to decrease moisture to a rate of 6%; for mainstream malts, temperature is increased up to a maximum of 105°C. With the heat treatment, the removal of water has been achieved, together with the formation of color and the production of aromatic compounds, formed by Maillard reactions between sugars and amino acids produced during the modification. The malt obtained must be stored for 2–4 weeks, in order to allow a uniform distribution of the residual moisture inside the seed. The following steps are the milling and the mashing of the malted grains: in milling, the grain is subjected to grinding to allow enzymes present in the seed to degrade the substances it contains. The mashing consists in mixing warm water (usually beginning at 50°C per 20 minutes) with malt flours, to allow the thermolabile β-glucanase to complete its activity. Increasing temperature to 65°C (1 hour) ensures the starch gelatinization and the action of amylase complex. The wort produced by conventional mashing has 20±25% starch left behind as non-fermentable digestion products called dextrins. Then, the wort is separated from the grains and boiled in order to achieve different goals: water evaporation, sterilization and enzyme inactivation, solubilization of α-acids of the hop and their isomerization to iso- α-acids, formation and precipitation of protein/polyphenol complexes, formation of reducing compounds, increase of the color wort, decrease of the wort pH, removal of DMS, removal of undesirable compounds of malt and hops. Then, the fermentation process takes place: the ales ferment more quickly than lagers, as the temperature needed by the ales is higher than that of the lagers. The fermentation lasts 3 to 14 days. Fermentation is the common denominator in the production of all alcoholic beverages; in brewing, it is determined by the presence of yeasts that transform sugars, derived primarily from malt, into ethanol. The last steps of the entire process are the maturation, the stabilization and the packaging. Maturation is the post fermentation step of brewing when beer is ready for filtration. Stabilization consists in the treatment of beer in order to extend its shelf-life: the principal technique is filtration (with kieselguhr or perlite) that is used to remove the residual haze precursors. By pasteurization or filtration, any microbial contamination is removed. Finally, the beer is packaged, either into can, bottle (glass or plastic), keg, cask, or bulk tank. The packaging process must be efficient, not only in terms of speed but also in terms of quality: no oxygen should be retained in the beer since it would cause the staling of the product (Bamforth, 2004).

1.3. Wheat beer

Wheat is often used for top-fermenting beers, in the form of malt, while it is rarely used in unmalted form. Since wheat malt has a high protein content and a high extract yield, the percentage that is used for the production of wheat beers is between 50 and 60% (in Italy up to 40%). This has a technological significance because it is known that high wheat malt can cause problems in the filtration stage due to its high protein content. Gluten, the characteristic protein of wheat, composed of a mixture of different proteins, notably gliadin and glutelin (instead of the hordein in barley), represents about 80% of the total proteins. For this reason, only some varieties of wheat are used for the production of beer, and winter varieties are the preferred ones as they are characterized by a lower protein content and a higher extract content than those of other seasons (Kunze, 2004).

Wheat has been grown for a long time as raw material for the production of malt and beer; it was firstly produced for food needs at least 8000–10000 BC (Toussaint-Samat, 1992; Pomeranz, 1998). However, greater attention has been paid to the production of barley beer than that of wheat beer. Wheat beer can be defined as a special beer that has developed in Germany in the late Middle Ages. By law, the German wheat beer is made up of at least 50% malted wheat and must be a top-fermented beer. General characteristic parameters for a wheat beer German style are as follows: original gravity (ºP) 1.047–1.056 (11.8–14 ºP), apparent extract/final gravity (ºP) 1.008–1.016 (2–4 ºP), alcohol by volume 4.9–5.5%, bitterness 10–15 IBU and colour of EBC 6–18.

Wheat beer has a long history and its production has significantly changed over the years. The histogram in Figure 1 shows the trend of German wheat beer production in the past 50 years (Global Malt Survey, 2010). It can be seen a constant and significant increase in consumption of wheat beer over the years with an almost doubling of production during the last 15 years, suffice to say that over 10 beers consumed, 9 are barley beers and the remaining one is a wheat beer.

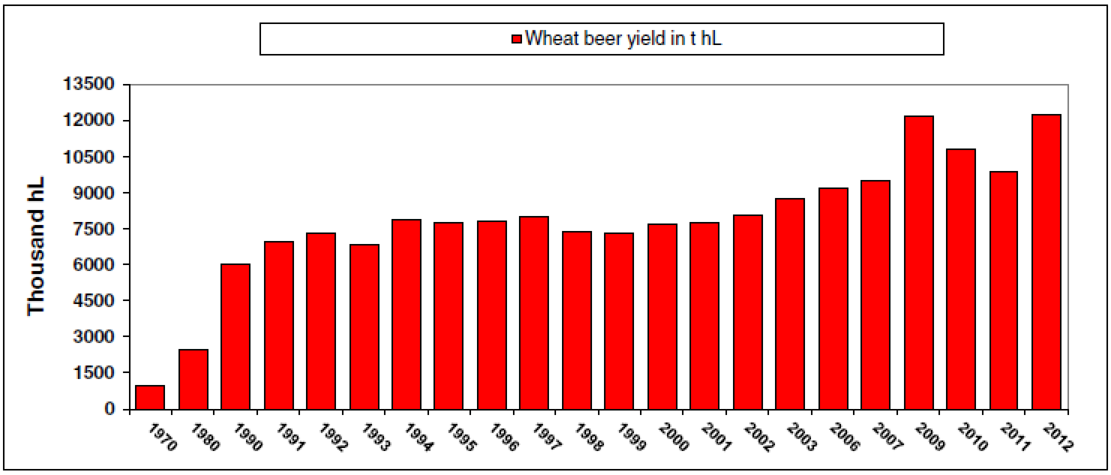

Figure 1. Trend of wheat beer yield from 1960 to 2012 (Faltermaier *et al.*, 2014).

The same trend has also been gained by wheat beers of other geographical origin, like the Belgian wheat beer (called “wit” in Flemish or “Bière blanche” in French). Unlike the German ones, Belgian wheat beers are brewed with unmalted wheat. In general, the amount of malted barley and unmalted wheat for wheat beer production is 60% and 40% respectively (Depraetere *et al.*, 2004). Belgian wheat beer can also be characterized by adding coriander and bitter orange peel, giving its lightly bitter and spicy notes. With the increase of wheat beer production, the demand for wheat has consequently increased. Actually, even if wheat production is very high (making it second after the cultivation of corn), the cultivated wheat is mostly aimed at bakery, in which the high protein content has a positive effect in bread making. This is not reflected in brewing because a high protein content determines a time-consuming clarification, filtration issues and problems of fermentation, as well as stability problems in the finished product. For this reason, the study of wheat as a raw material in brewing has been a little neglected from a scientific point of view. However, some brewers prefer to use a durum type of wheat flour. In fact, the use of a durum variety for beer production, when containing high molecular weight haze active proteins, can lead to a hazy white beer (Depraetere *et al.*, 2004).

1.3.1. Process technologies

The most commonly used techniques for the production of wheat beer are the following: the infusion process for an extent of 60% and the decoction process (typically a single decoction mash) for the remaining 40% (Hermann, 2005). The comparison between the two processes did not result in any difference in the composition of the aromatic components in the final beer produced (Hermann, 2005).

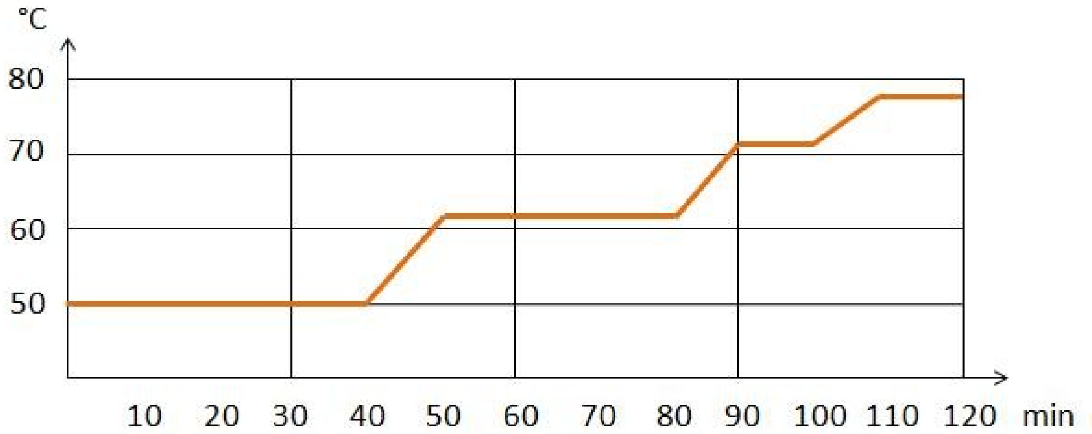

Figure 2. Infusion mashing (mashing-in at 50°C) (Kunze, 1999).

The infusion process is the traditional and simplest mashing process: the whole water/flour mixture is subjected to different temperatures (depending on the activity of the enzymes present in the mash, Figure 2) that are never so high to make the mashing mixture boil. The infusion mashing requires only one vessel which must be heated. As a consequence, during the whole process, the mash mixture is always kept in one vessel, reducing the amount of oxygen absorbed by the mash, thus limiting the oxidation of unsaturated fatty acids that alters the stability of beer flavour. As can be seen in Figure 2, a mashing temperature programmed infusion may provide a first rest of 35–40 min at 45–50°C. At these temperatures β-glucanases are still active. The temperature is then increased (1°C/minute) at 60–62°C for 30–40 min to promote the activity of β-amylase (saccharification phase) followed by a 1°C/minute increase rate, up to 70–72°C for 20–30 min to promote the activity of α-amylase (Kunze, 1999).

The infusion process is characterized by the following advantages:

- ease of automation;
- lower energy demand compared to the processes of decoction;
- ease of monitoring.

Nowadays, the infusion process is increasingly adopted by brewers that appreciate it for being easy to use (Kunze, 1999).

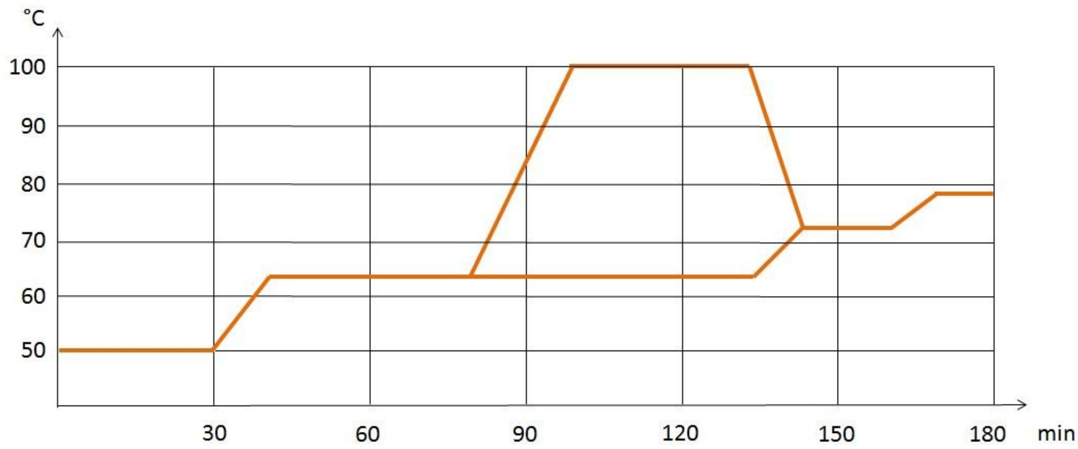

Figure 3. Single decoction process (Kunze, 1999).

In the decoction process, part of the mash is transferred to another vessel and boiled. Then it is pumped back in the mashing mixture that is thus heated up. There can be one to three boiled mashes that distinguish the single-, the two-, and the three-decoction mash processes. In theory, the single decoction mash is similar to the infusion process. In the single decoction process, malt is firstly mixed with water at a temperature of 38°C for 30 min. Temperature is then increased by 1°C/min to 50°C, held for 30 min, and increased again to 65°C for 35–45 min. Afterwards, the cooked mash is removed and boiled for 15–30 min, followed by a second mashing at a temperature of 75°C, after which a saccharification process was performed. After transferring the wort in the lautertun, the grist was sparged. During the mashing stage, vigorous agitation was avoided so as to minimize as much as possible the concentration of air inside the mash that would cause oxidation (Mascia *et al.*, 2014).

The decoction process causes the following effects:

- minor protein degradation of mash, caused by a more rapid heating ;
- wider starch gelatinization and liquefaction;
- greater extract from husks;
- increased concentration of melanoidins;
- removal top of dimethylsulphide (DMS);
- decrease of enzymes active in the total mash;
- higher yield of beers.

The boiling of part of the mash in the decoction process results in a higher energy demand, compared with infusion processes. Generally it requires an energy 10–20% higher than that needed for the infusion process (Kunze, 1999).

1.3.2. Volatile compounds of wheat beer

The aroma of beer is defined by the presence of several volatile compounds, such as esters, alcohols and carbonyl compounds (aldehydes and ketones). More than 600 volatile compounds in beer are known, and some types of beer have been extensively studied from a perspective of volatile component, with a characterization in terms of aroma. For example, in lager beers, it has been found that esters (e.g., 3-methylbutyl acetate and ethyl hexanoate), alcohols (e.g., 3-methylbutanol), dialkyl sulfides (e.g., dimethyl sulfide), and short-chain fatty acids (e.g., butanoic acid) are essential for the aroma. This has been proven through the calculation of the odor activity values (OAVs; ratio of concentration to odor threshold) of 239 volatile compounds. On the contrary, the studies on wheat beers are mainly based on optimization or on the variation of technological procedures, and little is known about the volatile component since only a few volatile compounds, particularly some metabolites of yeast, like higher alcohols, esters and diacetyl, were quantified. Since most of the aroma compounds of wheat beers are still unknown, the contribution describing the flavor profile of wheat beers is little: in a work on Bavarian wheat beers about 30 various compounds were studied in regards to their contribution to the aroma of beer (Langos *et al.*, 2013).

1.3.2.1. Esters and alcohols

The most important aroma compounds produced by yeast are esters. Esters are present in a very low concentration (in trace) compared to other fermentation metabolites but, because of their high volatility and low odour threshold (Meilgaard, 1975; Saison *et al.*, 2009), they are responsible for the aroma of the beer. It is therefore important that the concentration of esters is balanced for not having negative effects on beer aroma, such as a bitter predominating over a fruity taste, when esters are overproduced. Thus, brewers have to maintain the optimal conditions for esters production, to obtain a balanced-aroma beer (Pires *et al.*, 2014).

Esters are mainly synthesized during the primary fermentation by enzymatic chemical condensation of organic acids and alcohols. Their synthesis takes place in the cytoplasm of the brewing yeast but they easily move outside the cell because of their lipophilic character.

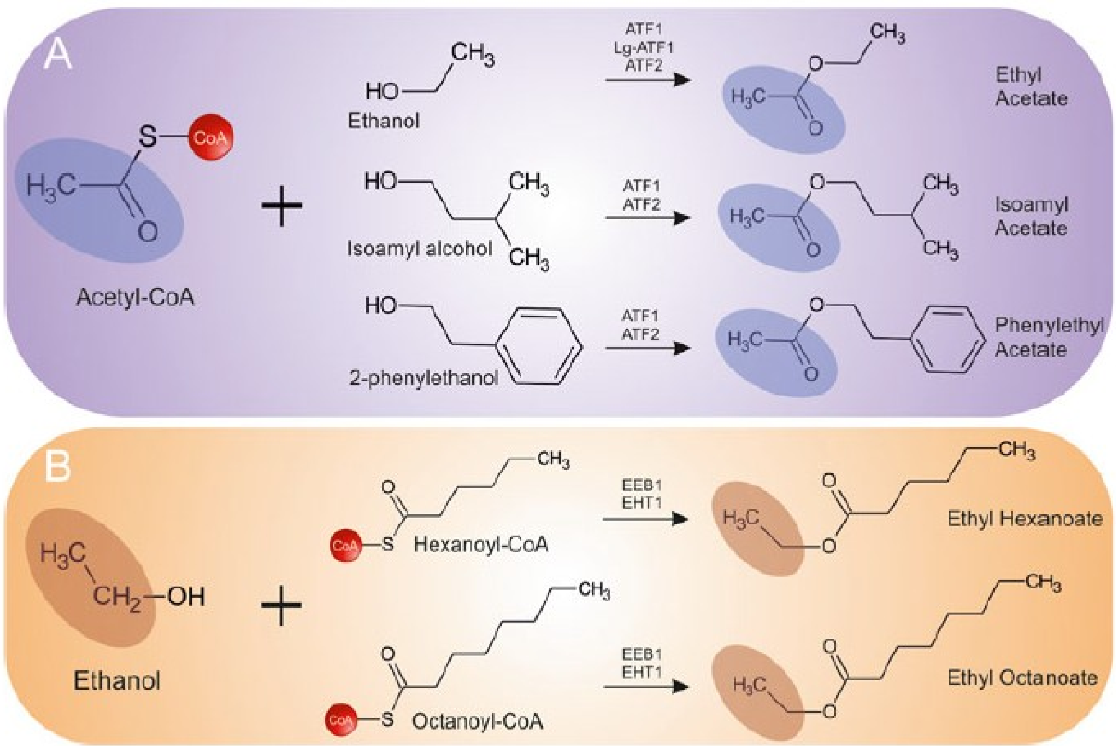

Figure 4. Scheme of reactions leading to the formation of acetate esters (**A**), and MCFA ethyl esters (**B**). The principal genes involved in each reaction are shown over the reaction arrow (Pires *et al.*, 2014).

Two different groups of esters can be defined based on their chemical structure: the acetate esters and the medium-chain fatty acids (MCFA) ethyl esters. Acetate esters are formed by reaction of acetyl-CoA with ethanol or a higher alcohol (Figure 4A), while MCFA ethyl esters are formed by reaction of ethanol and long chain acyl-CoA (Figure 4B).
Despite beer contains about 60 different esters, only six (Figure 5) are considered to be of great aromatic importance, each one conferring a distinctive character to beer aroma: ethyl acetate, which confers a solvent-like aroma; isoamyl acetate, which gives a banana aroma; isobutyl acetate, which confers a fruity aroma; phenyl ethyl acetate, which produces a rose and honey aroma; ethyl hexanoate, which gives a sweet apple aroma and ethyl octanoate, which confers a sour apple aroma (Pires *et al.*, 2014).

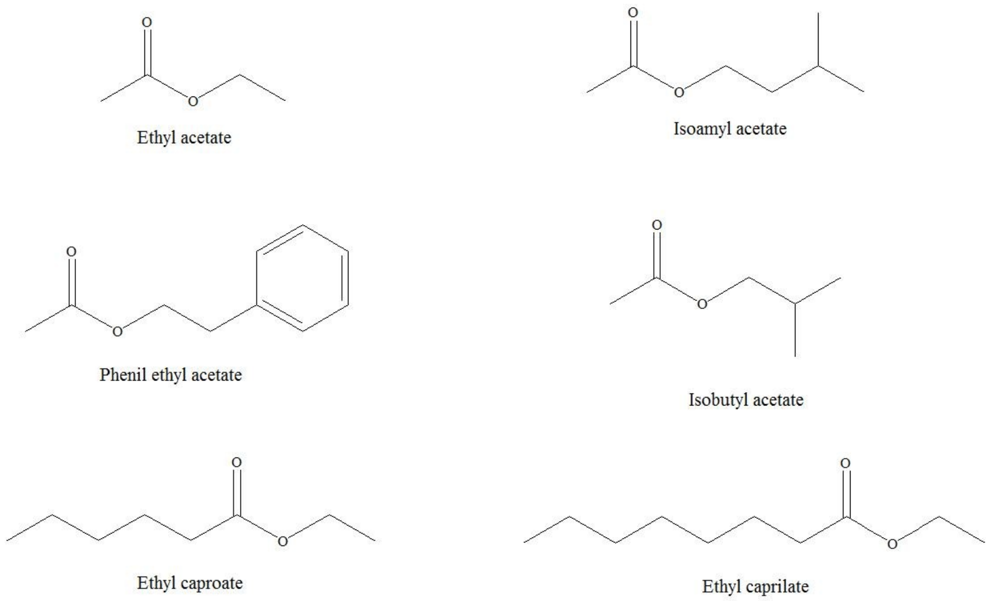

Figure 5. The most important aromatic esters in beer.

Besides, the higher alcohols (also called fusel alcohols) are the organoleptic compounds with the highest concentration in beer. Higher alcohols are formed as by-products of the catabolism of some amino acids present in wort (valine, leucine, isoleucine, methionine, and phenylalanine), in an enzymatic chain reaction pathway that was proposed by Ehrlich (Ehrlich, 1907) (Figure 6). Firstly, amino acids are converted to glutamine in a transamination process, forming α-ketoacids as by-product. Only a part of the α-keto acids is redirected into central carbon metabolism, whereas the remaining is decarboxylized to aldehydes that are finally reduced to form higher alcohols.

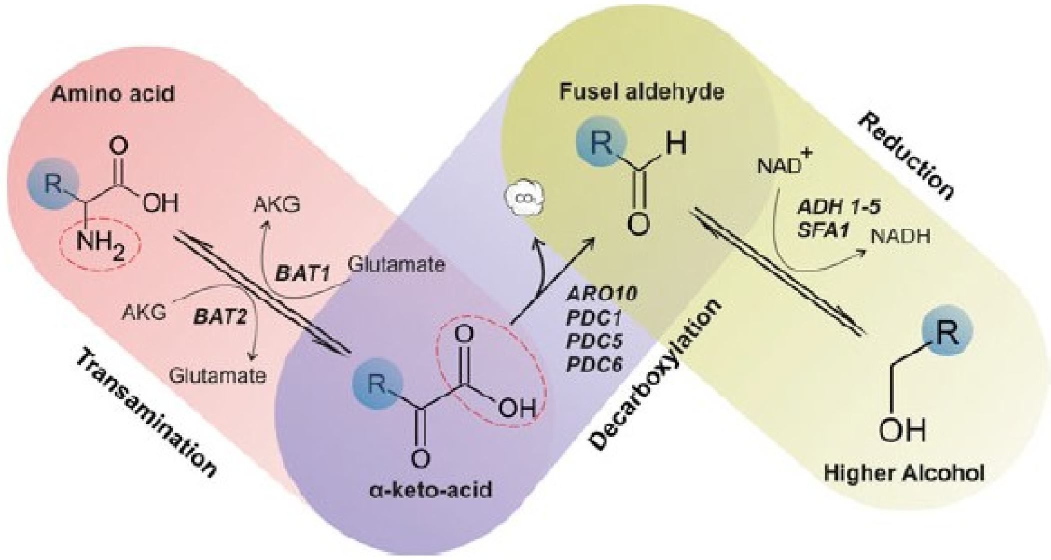

Figure 6. The Ehrlich pathway for higher alcohol synthesis (Pires *et al.*, 2014).

The transamination and reduction reactions are reversible while the decarboxylation reaction is an irreversible process. Ehrlich noticed structural similarities between the active amyl alcohol and isoleucine and between isoamyl alcohol and leucine. This made him suppose that the amino acids present in wort were involved in the synthesis of higher alcohols. Ehrlich investigated this relationship by adding the two amino acids in the yeast fermentation mixture, observing an increased concentration of the related higher alcohols. Therefore, Ehrlich proposed a pathway for higher alcohol synthesis, in which an amino acid is enzymatically converted to the correspondent higher alcohol, developing carbon dioxide and ammonia. Since ammonia was not found in beer, Ehrlich supposed it was incorporated into yeast proteins. Not all the amino acids that follow the Ehrlich pathway come directly from the wort but they can also be formed by *de novo* biosynthesis through the metabolism of the carbohydrates of the wort (e.g. fructose, glucose and maltose). Figure 7 shows the main metabolic routes in the brewing yeast cell that lead to the formation of higher alcohols and esters.

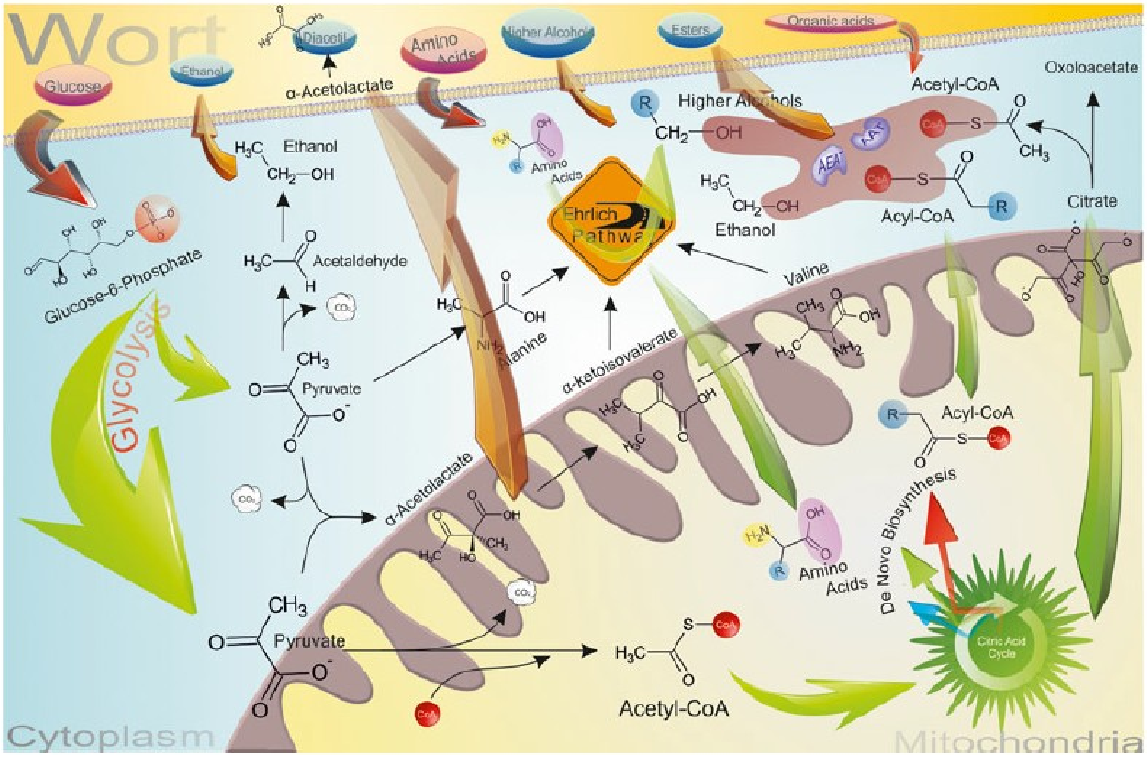

Figure 7. Summary diagram of the reactions that occur when the yeast is inserted in the fermenting wort, with the formation of higher alcohols and esters (Pires *et al.*, 2014).

1.3.2.2. Carbonyl compounds

Carbonyl compounds are responsible for the stale flavor in beer. In particular, aldehydes are the major deterioration agents which negatively affect beer aroma and flavour (Moreira *et al.*, 2013). Acetaldehyde is the first one to be reported as a substance that affects beer flavour during aging (Engan, 1969) but the most studied aldehyde is (E)-2-nonenal, since it was found to be responsible for the taste of cardboard that is present in some aged beers (Palamand *et al.*, 1969). Nowadays, it is universally accepted that a stale flavor is caused by a myriad of flavour compounds that are the result of different formation and degradation reactions. The precursors of most of the carbonyl compounds present in beer come from the raw materials; the reactions take place during the pre- and post-fermentation phases of the production process (Ojala *et al.*, 1994) but in particular during the beer aging. The main reactions for the synthesis of aldehydes and ketones are:

- Oxidation of higher alcohols;
- Strecker degradation;
- Aldol condensation;

- Oxidation of unsaturated fatty acids;
- Maillard reactions;
- Degradation of bitter acids;
- Autoxidation of aldehydes.

Among these reactions the following mechanisms can be mentioned (Moreira *et al.*, 2013):

Strecker degradation

The compounds that are subjected to the Strecker degradation are amino acids, that form aldehydes. The reaction takes place between an amino acid and an α-dicarbonyl compound, catalysed by Fe and Cu ions. The Strecker degradation involves transamination to form an α-ketoacid which is decarboxylised to aldehyde (Figure 8). The aldehyde formed has one carbon less than the starting amino acid (Vanderhaegen *et al.*, 2006). Besides, some authors state that Strecker degradation is possible only with high amounts of amino acids but not with the typical concentrations of amino acids in beer (±1 g/L) (Thum*et et al.*,1995). Important aldehydes formed by Strecker degradation are acetaldehyde, phenylacetaldehyde, 2-methyl-propanal and 3-methylbutanal.

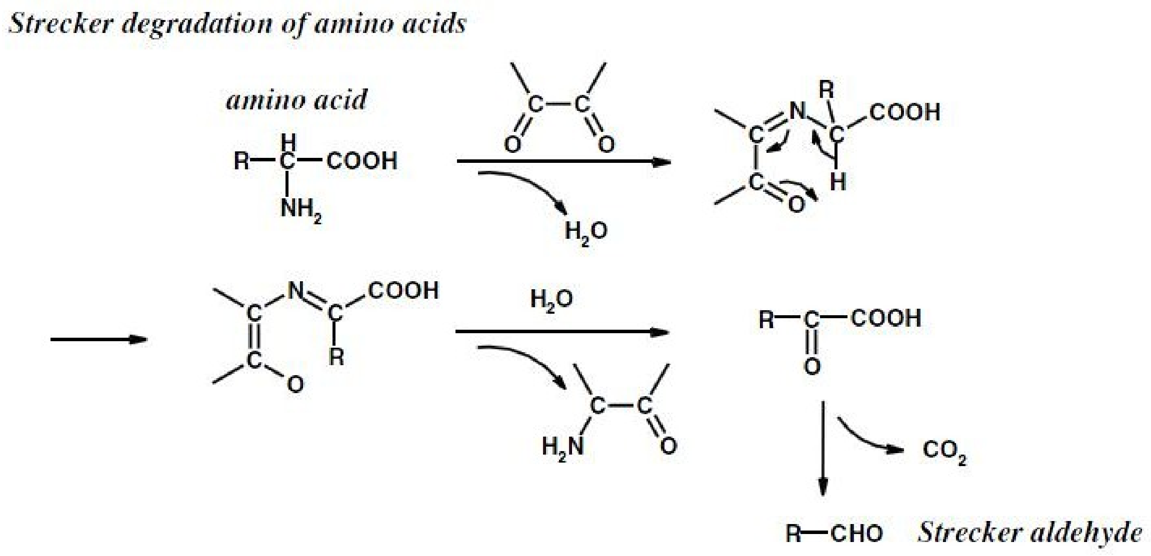

Figure 8. Pathway for Strecker degradation (Vanderhaegen *et al.*, 2006).

Aldol condensation

Aldol condensation takes place between carbonyl compounds with an amino acid as catalyst. The reaction is possible even under the mild conditions that exist in beer during storage. Figure 9 shows the aldol condensation of acetaldehyde with heptanal to form (E)-2-nonenal, through an imine intermediate. Aldol condensation produces flavour carbonyl compounds that are more active than their precursors.

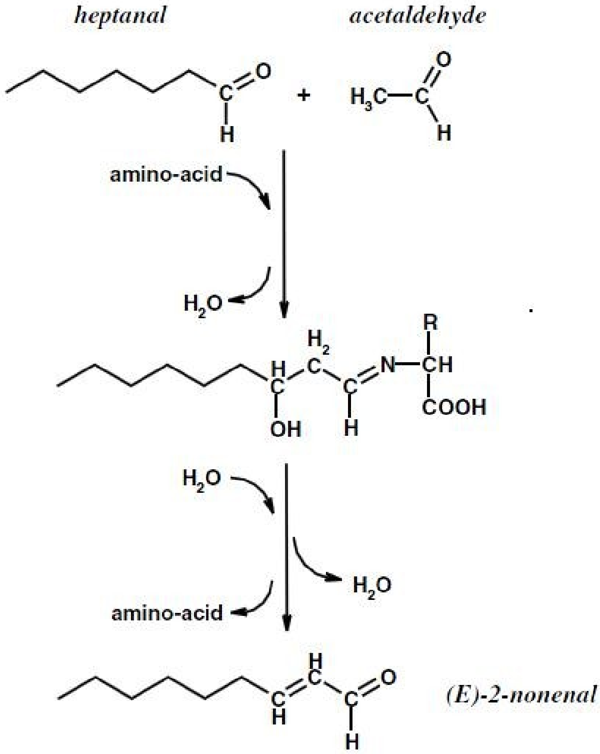

Figure 9. Formation of (E)-2-nonenal in beer according to Hashimoto *et al.* (1975).

Degradation of bitter acids

The bitter acids present in hops (iso-α-acids, α-acids and β-acids, Figure 10) can be degraded resulting in a decrease of beer bitterness but enhancing beer aging flavor by formation of other substances. The degradation pathway has not been properly established yet. Some of the proposed schemes for iso-α-acids degradation involves radical oxygen species (Kaneda *et al.*, 1989), (De Cooman *et al.*, 2000) but other works indicate that the electron acceptor compound is not necessarily an oxygen specie (Huvaere *et al.*, 2003). The radicals formed (oxygen- and carbon-centered radicals) lead to different products that are characterized by the lack of the tricarbonyl chromophore present in the starting molecule.

The substances obtained by degradation of bitter hops are carbonyl compound with various chain lengths (i.e. C3 to C11 2-alkanones, C2 to C10 alkanals, C4 to C7 2-alkenals and C6 to C7 2,4-alkedienals) (Hashimoto *et al.*,1979). The molecules that were identified as oxidation products were acetone, 2-methyl-propanal, 3-methyl-butan-2-one, 4-methyl-pentan-2-one and 2-methyl-3-buten-2-ol (Hashimoto *et al.*, 1975), and 2-methyl-propionic acid, 2-methyl-butyric acid and 3-methyl-butyric acid (Williams *et al.*, 1979) that are the precursor acids of staling esters.

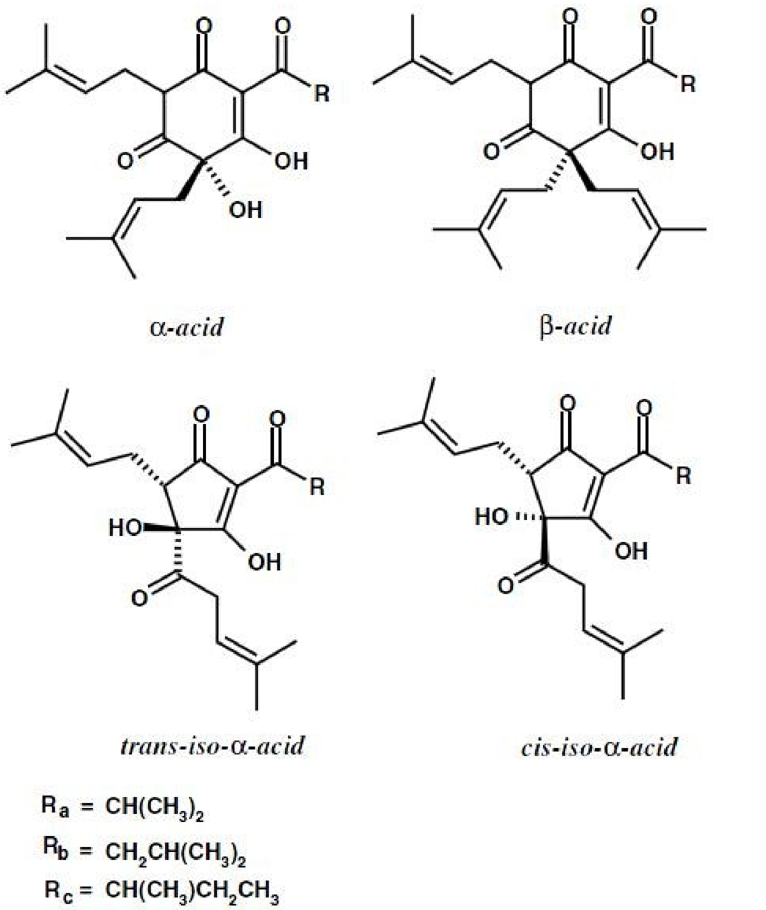

Figure 10. Chemical structure of the most important bitter acids in beer (Vanderhaegen *et al.*, 2006).

Maillard reaction

Heterocyclic compounds found in aged beers are formed by Maillard reaction. The known Maillard products in aged beers, such as furfural and 5-hydroxymethyl-furfural (5-HMF), appear to be under the threshold of flavor and therefore, the studies on these compounds are scarce. Maillard products are the responsible ones of the development of sweet, bready and wine notes in aged beer (Bravo *et al.*, 2001). Quantitatively, 5-hydroxymethyl-furfural (5-HMF) is the most important heterocyclic compound formed during beer aging. The mechanism of the Maillard reaction leading to 5-HMF is shown in Figure 11: the reaction between the carbonyl group of a hexose and the amino group of an amine leads to the formation of an imine. Through a subsequent Amadori rearrangement, a more stable molecule, the Amadori product 1-amino-1-deoxyketose is formed. At the pH of beer, Amadori product enolizes to 1,2 enaminol which, after removal of an amine group, is converted to an α-dicarbonyl(3-deoxy-2-hexosulose). After water removal and conversion into the cyclic form, 5-HMF is obtained. When starting form a pentose instead of a hexose, furfural is formed (Vanderhaegen *et al.*, 2006).

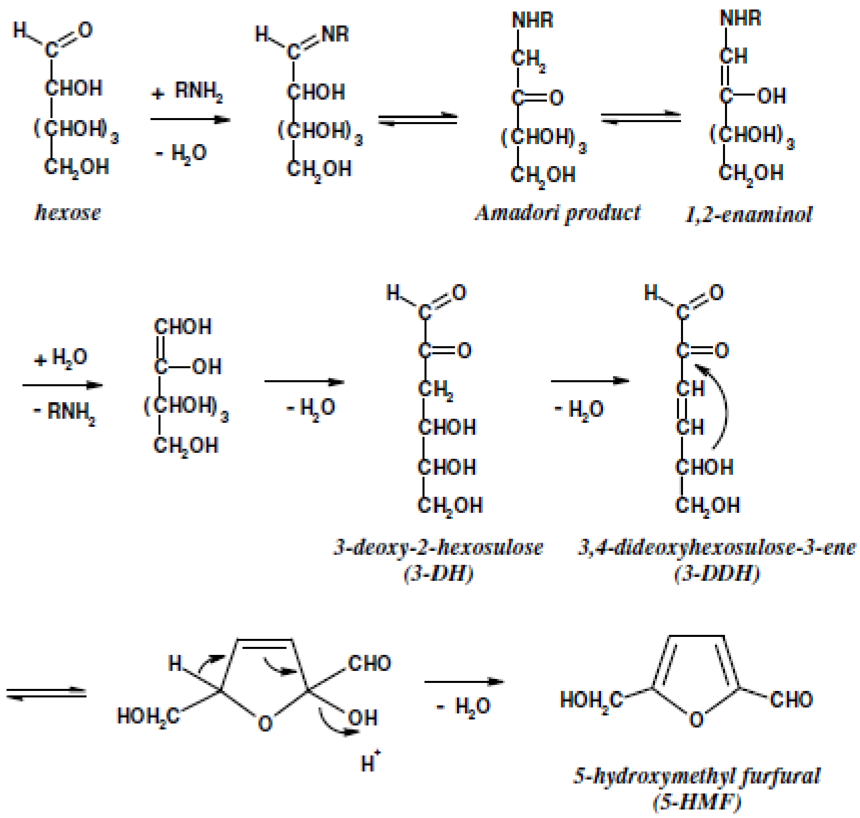

Figure 11. Formation of 5-hydroxymethyl furfural by Maillard reaction (Vanderhaegen *et al.*, 2006).

1.3.3. Sensory evaluation

Sensory analysis techniques are a set of methods that can be applied to every food. Each food can be described by different attributes which have to be measurable, objective, and related to the chemical, physical and technological characteristics of the food analyzed.
Sensory analysis techniques applied to beer were developed through a collaborative effort with the European Brewery Convention, the Institute of Food Technologists (Chicago) (IFT), the American Society for Testing and Materials, and the International Organization for Standardization (ISO).
Beer tasting is a very complex sensory experience. The reason is related to a production technology that requires several stages of production, ingredients and additives (Società Italiana di Scienze Sensoriali, 2012).
Sensory analysis of beer at all stages of production is needed, therefore sensory methods have to be applied both to the finished beer and to the raw materials.
The application of sensory analysis is important in quality control, research, product development and it is also a way to solve possible problems in technological process. Sensory analysis uses assessors as measuring instrument. Since assessors are variables and prone to bias, it is necessary to train a panel and repeat the test several times in order to have reliable results. Finally the data must be treated statistically. For this reason the test design should be well detailed as the main purpose is to control the variability and to reduce to a minimum the bias (ASBC, 2014). The steps to follow in the sensory analysis are the selection of a problem, the formulation of an idea and the selection of a practical test. In fact it may be insignificant to make a preference test without having firstly demonstrated that there exists a significant difference between the samples. Generally the tests have two main aims, one is to describe the product and the other is to distinguish between two or more products. Before one begins with the practical tests, a statistical plan should be done, especially when more than one session is needed, due to the number of samples to be analyzed. Typically, 3 or 4 samples are being used during a session of a descriptive test, while no more than 6 samples are being used in the case of the duo-trio or triangular test. These numbers may be increased in the case of visual tests, in which visual parameters such as foam, color, etc. are determined (ASBC, 2014).
Beer is characterized by a very complex technology, and a large number of products can be used as ingredients. For this reason different types of beer are present on the market, differing for foam, colour, odour, flavour and tactile sensations. The "flavour wheel"

(figure 12) defined by Meilgaard *et al.* (1979) and later supplemented by Walker and Westwood (1992), Coghe *et al.* (2004) and Schmelzle (2009) represents such sensory variability.

In the beer wheel, as indicated by Meilgaard *et al.* (1979), descriptors are grouped into broader categories based on their similarities, in an order that follows the normal sequence of evaluation: firstly those that relate to the fragrance sensations perceived by olfaction and later those relating to flavour and tactile sensations in the mouth.

Besides, the distinction between positive and negative descriptors, is not reported in the wheel. Moreover, some descriptors, such as acetaldehyde, ethyl acetate, and plastic trash bag (usually considered as defects) are placed in the wheel under positive aromatic classes (Società Italiana di Scienze Sensoriali, 2012). In fact sensory analysis can be used also to verify that the drink has no defects or alterations.

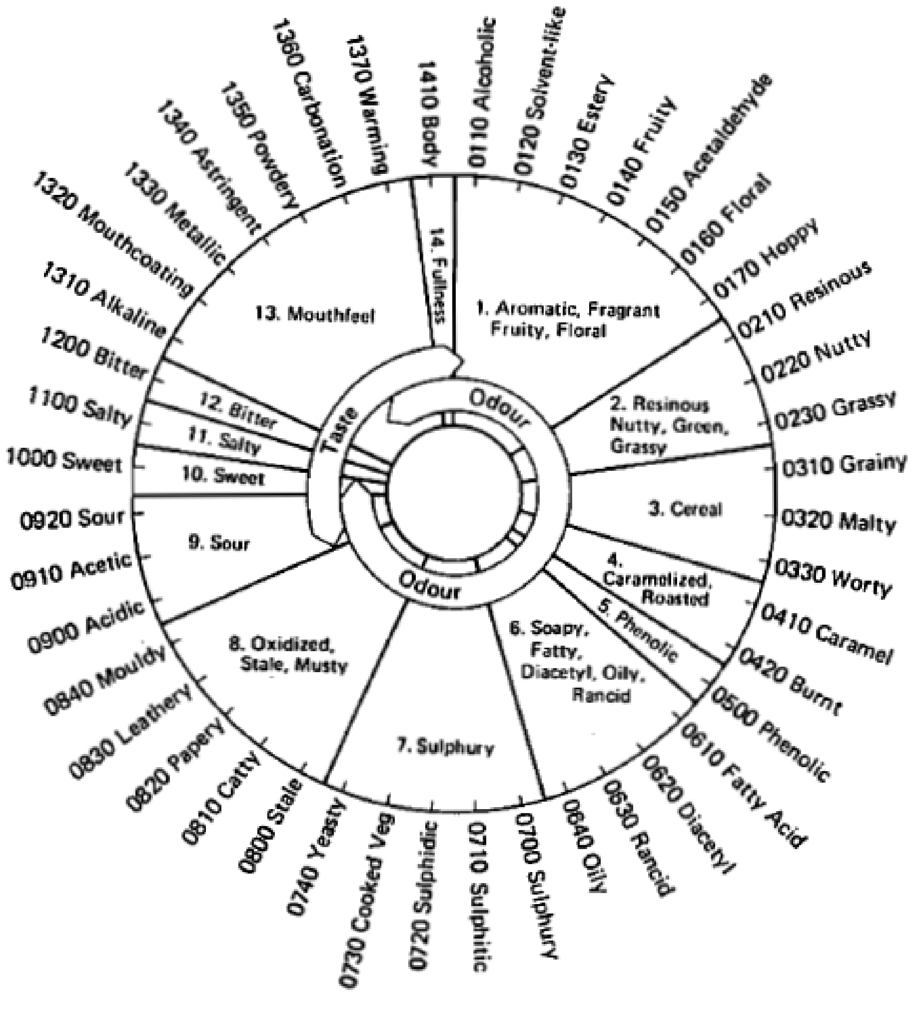

Figure 12. Flavour wheel (Meilgaard *et al.*, 1979).

2 AIM OF RESEARCH

In describing what is the purpose of this doctoral thesis project, it should be highlighted why the attention was focused on a craft wheat beer brewed with wheat "Cappelli" grown in Sardinia. When speaking of craft beers, one refers to small production of beer produced by small breweries, whose objective is to produce traditional beers that are able to compete with the industrial beers, pointing the interest on their quality and diversity (Brewers Association, 2013). Craft beers are commonly indicated as beers that are not filtered, not pasteurized and re-fermented in bottle; therefore the choice of yeast is very important in the craft beer production (Bokulich *et al.*, 2013; Canonico *et al.*, 2014). One of the differences between the large brewing companies and the micro-breweries is that the firsts use yeasts produced in-house whereas the latters use commercial strains (Quain, 2006). One of the criteria used for the selection of the industrial strains for beer production lies in the ability of the strains to rapidly and completely ferment the available carbohydrates (Batistote *et al.*, 2006).

So it is easy to understand the importance of the characterization of strains of *S. cerevisiae* isolated from food matrices different from beer, as this might be a valid approach of the selection of a starter for beer production. In this thesis it was assessed the feasibility of using *S. cerevisiae* yeast strains, isolated from craft sourdough, as starters of the production of craft beers (Marongiu *et al.*, 2014). The sourdough, indeed, can represent an alternative source for the isolation of starter yeasts for brewing. The environment of sourdoughs is dominated by different yeast species, among which is the *S. cerevisiae* (Gobbetti *et al.*, 1994). Strains of this specie showed an efficient uptake and utilization of trehalose that share common transporters with maltose (Stambuk *et al.*, 1996). Thus, these strains may be able to ferment maltose, the main carbohydrate of beer (Gjertsen, 1953).

The goal of this thesis was to test the production of a local craft beer by the selection and use of varieties of wheat and barley grown in Sardinia, by the choice of autochthonous yeasts isolated by sourdoughs that best could characterize the beer, and by studying the shelf life of the beers produced.

Using the commercial wheat beer as a term of comparison (control sample) there were developed 3 main points related to each other:

1) Preliminary characterization of an Italian craft durum wheat beer (Mascia *et al.*, 2014): currently, nothing or little is known about wheat beers and their volatile

component. The first goal was the characterization of an Italian durum wheat beer, and the comparison with two other wheat beers, one produced in Germany and one in Czech Republic. The originality of this point lies in the fact that this is the first time that an Italian wheat beer, and in particular a Sardinian craft durum wheat beer has been characterized.

2) Is it possible to create an innovative craft durum wheat beer with sourdough yeasts? A case study: this possibility was tested by producing two local craft beers (brewed with 20% of an old variety of unmalted durum wheat grown in Sardinia) in which two different yeasts were used: a commercial one, normally used by brewers for wheat beers, and an autochthonous one, selected by the Microbiology section of the Department of Agraria (University of Sassari) from a Sardinian sourdough (Marongiu *et al.*, 2014). The possible use of an autochthonous yeast could provide an additional element of originality to the existing production, thus creating a further link with the territory that could have major repercussions from the economic point of view. This is important to create the basis for the expansion of export markets outside the region, trying to enhance the originality of the Sardinian products.
3) Study of the shelf life of the three beers produced with three different yeasts, one commercial and two autochthonous. The objective was to study the shelf life of the three beers over six months ,and to obtain relevant information concerning the decay quality as a function of two different storage temperatures. Half of the beers were retained at a temperature of 8°C, normal cold storage temperature, and the other half at a temperature of 28°C, shelf temperature. The main objective was to test the possibility to expand the market for craft beers outside the region.

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4 CHAPTER I

Preliminary characterization of an Italian craft durum wheat beer

Research article

Institute of Brewing & Distilling

Received: 30 May 2014 Revised: 30 July 2014 Accepted: 31 July 2014 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jib.176

Preliminary characterization of an Italian craft durum wheat beer

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In recent years, there has been increasing interest in the production of beers brewed using raw materials of local origin. Many micro-breweries have been established in Italy, particularly in Sardinia, where some have started to use Sardinian wheat to produce a beer that has a regional connection. The most widespread wheat cultivar used in Italy has been Senatore Cappelli, which is still grown in Sardinia and in some other regions. Bread produced with this type of wheat is characterized by a high protein content and good sensory properties. Owing to its origin, this cultivar is being considered for use in beer production. In this work, a Sardinian durum wheat beer was compared with two other wheat beers brewed in Europe (Germany and the Czech Republic). Standard beer chemical analyses, along with volatile and sensorial profiles, were used to characterize the Sardinian craft durum wheat beer. Copyright © 2014 The Institute of Brewing & Distilling

Keywords: durum wheat; craft beer; volatile and chemical compounds

Introduction

Beer is produced mainly from barley, hops, water and yeast, and beer quality is influenced by the characteristics of the raw materials, knowledge of which is essential for determining the correct technological processes. Barley is the most widespread raw material used for brewing owing to its high content of starch, which, once degraded by enzymes, is the main source of carbohydrate for yeast during fermentation. In addition, after threshing and processing to malt, the husk still adheres to the grain, thus forming a wort filtration layer that is necessary for beer production. In the brewing industry, it is common to use unmalted cereals such as maize, rice, sorghum, barley and wheat, or their derivatives, as a source of starch *(1)*.

Despite different varietal properties of wheat, brewers have not specified wheat quality characteristics for brewing and wheat varieties are distinguished primarily by factors such as winter or spring variety, protein content and the hardness or softness of the grain *(2)*. Some researchers claim that wheat used for brewing should be of a soft variety, because it is more easily milled, leading to maximum yield, with a protein content of up to 11% (w/w) and a mealy or floury endosperm *(3)*.

Baetslé also suggests the use of a soft variety, for beer production, as reported in Depraetere *et al*. *(2)*. In contrast, the use of a durum variety for beer production, when containing high-molecular-weight haze active proteins, can lead to a hazy white beer. Some brewers prefer to use durum wheat to obtain this particular effect *(2)*.

In 2012, Italian beer production was ~13.5 million hL, representing about 75% of Italian consumption (17.6 million hL, corresponding to 29.5 L per capita). Beer consumption in Italy is at the bottom of the European ranking scale, with an average that is <40% of the European average, and three to five times lower than that of countries with the highest consumption (Czech Republic with 144 L per capita, Austria with 108 L per capita and Germany with 105 L per capita) *(4)*.

In Italian law, the term 'beer' is reserved for a product obtained by alcoholic fermentation of a wort made with either malted barley or wheat (or a mixture) and water, using strains of *Saccharomyces carlsbergensis* or *S. cerevisiae*. Adding hops or their derivatives gives the bitter taste that is characteristic of beer. Barley and wheat malt may be substituted with other grains, or sugary and starchy raw materials, to a maximum of 40% (w/w), calculated on the dried wort extract *(5)*.

In Sardinia in recent years, several micro-breweries producing craft beers have been established. A micro-brewery is characterized by limited beer production, and is focused on using the best ingredients for brewing high-quality beers *(6)*. Where possible, craft beer is expected to show typical characteristics and originality. Some researchers define craft beer as a product that, in most cases, is not filtered or pasteurized, maintaining its original sensory characteristics. For this reason, craft beer is more susceptible to microbial contamination than industrial beers, resulting in deterioration such as turbidity, production of undesirable aromatic compounds and high acidity *(7)*. Durum wheat (*Triticum turgidum* L. var. *durum*) is produced worldwide, occupying 20–30 million hectares; more than half of this area is in the Mediterranean region. It is traditionally the main crop in Italy, especially in southern areas, such as Sicily and

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Sardinia *(8)*, and is of economic importance mostly for the production of pasta and different types of bread in various areas of the world *(9)*.

The most widespread durum wheat cultivar in Italy is traditionally Senatore Cappelli *(10)*, which is still grown in some regions. Mastromatteo *et al*. *(11)* reported that the Cappelli cultivar used for the production of bread has excellent textural and chemical properties and bread produced from this wheat is characterized by a high protein content and soluble fibre, and good sensory properties. Owing to the origin of this wheat variety, it has been tested for use in beer production.

The objective of this study was to compare an Italian durum wheat beer with two other beers produced using wheat derived from other countries. The importance of this work lies in the fact that, until now, wheat beers have been poorly studied, with little attention being paid to volatile components *(12)*. Moreover, to our knowledge, our study is the first one in which an Italian (and particularly Sardinian) wheat beer is characterized.

Material and methods

Beer samples

The beer samples were of a commercial wheat type, produced and bottled in three different countries: Italy (Sardinia), the Czech Republic and Germany. The Sardinian beer was unfiltered and unpasteurized, while the Czech and German beers were unfiltered but pasteurized. The Italian beer was a craft beer, while the other two were industrial beers. The beer compositions were 50% malted barley and 50% malted wheat for the German and Czech beers, while the Sardinian beer was made from 60% malted barley and 40% wheat, of which half was commercial malted wheat and half was unmalted 'Cappelli wheat' grown in Sardinia.

The mashing technique used for the Czech and German beers was decoction, while for the Italian beer it was infusion. In the decoction process, the temperature is increased by removing part of the mash and boiling it. The temperature of the total mash is increased to a higher temperature by mixing the heated mash with the remainder. This process can be repeated several times but, in the case of the Czech and German beers, only one decoction step was used (a single decoction mash), which is very close, in principle, to the infusion process.

The Czech beer was prepared by a single mash process, which consisted of several steps: malt was mixed with hot water at 38°C and the temperature was held for 30 min, then increased to 50°C at a rate of 1°C/min and maintained for 30 min. The temperature was then increased to 65°C and held for another 30–45 min. The cooked mash was removed and boiled for 15–30 min, after which a second mashing at 75°C was performed, followed by saccharification. The mash was transferred to the lauter tun where the grist was sparged. During temperature-programmed mashing, vigorous mixing was avoided to minimize entrainment of air with consequent oxidation of mash constituents.

The infusion method is generally used in Italy for the production of wheat craft beers. This method is carried out in a single mash mixing vessel. In the case of Sardinian beer, the mashing plan followed several steps. The malt was mashed at 50°C and the temperature was held for 20 min. Then the temperature was increased to 62°C at a rate of 1°C/min and maintained for 40 min. The temperature was then increased to 72°C and held for 20 min. Finally, the temperature was increased to 78°C and held for 10 min.

Typically, wheat beers are top-fermented, and all the three beers characterized in this paper were fermented with *S. cerevisiae* strains. In particular, the Sardinian beer was fermented with the Safbrew S33 commercial yeast strain (Fermentis, Lesaffre Italia S.p.A., Italy).

Reagents

The hop extract standard, calibration standard ICS-I3, was used for the *iso*-α-acid determination, and was obtained from Labor Veritas, Zurich. HPLC-grade methanol and *ortho*-phosphoric acid were obtained from Merck (Germany) and Riedel–de Haën (Germany), respectively. Milli-Q water was produced using a Milli-RO 5plus (Millipore, USA) apparatus. Helium 5.0 quality gas (Messer, Czech Republic) was used for the GC/MS analysis.

Ethanol, acetaldehyde anhydrous, dimethyl sulphide, ethyl acetate, propyl acetate, 2-methylpropyl acetate, ethyl butyrate, butyl acetate, 3-methyl-butyl acetate, ethyl hexanoate, ethyl-2-hydroxy propanoate, 2-phenyl-ethyl acetate, ethylhexanol, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl hexadecanoate, *n*-propanol, 2-methylpropanol, 2- and 3-methylbutanol, furfurylalcohol, 2-phenylethanol and *n*-butanol internal standard, were purchased from Sigma-Aldrich (USA) and were >99% purity.

Physical–chemical analyses

Beer alcohol content was measured on an Alcolyzer (Anton Paar GmbH, Austria). Standard beer analyses and determination of colour, bitterness, total polyphenols and pH were carried out according to EBC methods 9.6, 9.8, 9.11 and 9.35 *(13)*. Foam stability was evaluated according to MEBAK method 2.18.2 *(14)*, using the NIBEM-TPH foam stability tester with the Inpack 2000 Sampling Device, type ISD (Haffmans, Holland). Titration acidity was carried out using an in-house method of the Research Institute of Brewing and Malting in Prague *(15)*. Carbon dioxide was determined according to the MEBAK method 2.26.1 *(16)*.

HPLC determination of beer bitterness

C18 SPE columns (Strata-X 33 µ polymeric reversed-phase 60 mg/3 mL, Phenomenex) were activated with 5.0 mL of methanol and 5.0 mL of water. Then 5 mL of degassed beer sample was applied. The column was washed with 2.5 mL of a solution of 5% methanol, and then *iso*-α-acids were eluted with 2.2 mL of methanol *(17)*. HPLC analysis of *iso*-α-acids was performed on an Agilent 1100 Series with an autosampler, a quaternary pump, a thermostat and an Agilent 1200 series diode array detector.

Separations were carried out on a Macherey Nagel EC 12J/4 Nucleodur 100-5 C18 EC column at a temperature of 35°C *(17)* and flow rate of 1.0 mL/min. The detector was set at 270 nm to quantify *iso*-α-acids, and at 314 nm to quantify α- and β-acids. The mobile phase was 100% methanol (A) and methanol–water–*ortho*-phosphoric acid–EDTA (750:239:10:1, B), with gradient elution programmed as follows: 0 min, 0% A, 100% B; 0–20 min, 0% A, 100% B; 20–20.1 min, 35% A, 65% B; 20.1–30 min, 35% A, 65% B; 30–30.1 min, 0% A, 100% B; 30.1–35.0 min, 0% A, 100% B. For quantification of *iso*-α-acids, a dicyclohexylamine salt of *trans*-*iso*-α-acid ICS-I3 complex

[62.3% (w/w) *iso-α*-acids; Labor Veritas, Zürich, Switzerland] was used as an external standard.

GC-FID profiling of alcohols and esters

Determination of volatile alcohols and esters in beer was performed by headspace sampling using a Carlo Erba 5300 Mega gas chromatograph equipped with an autosampler according to EBC method 9.39 *(13)*. Analytes were separated on a conventional 60 m × 0.32 mm i.d. fused silica capillary column (J&W Scientific DB i.d-WAX) with 0.25 μm film thickness. The GC column was maintained at 45°C for 1 min, ramped at a rate of 10°C/min to 135°C and then held at this temperature until the end of the analysis. Temperatures of the injector and the flame ionization detector were set at 110 and 250°C, respectively. Helium 5.0 quality carrier gas was used, with a column head pressure of 150 kPa at 80°C. The Clarity software program (DataApex, Czech Republic) for identification and quantification of volatiles was used. Peak areas were normalized using *n*-butanol as an internal standard and the concentrations of volatiles were determined using calibration factors obtained by the analysis of known mixtures.

Sensory analysis

Sensory analysis was performed using a trained panel of 15 assessors. Three beers were randomly presented in one session to the panellists, who performed a simple descriptive test as reported in the ASBC method *(18)*. A sensory profile was carried out, quantifying the sensory attributes selected, relative to the odour and taste, by giving them an intensity score from 1 to 5, where 1 indicated a very weak intensity and 5 a very strong intensity. Sensory attributes were as follows: for odour, total intensity, guaiacol and banana; for taste, acidity, sweetness, bitterness at the first draught and after swallowing the beer, bitter intensity and character; for mouth/feel sensations, freshness (CO_2) and fullness. The score card used for lager beers was modified for wheat beers *(19)*. The choice of two attributes, phenolic odour note and banana, was due to the fact that these are typical notes characterizing wheat beers, as reported by Langos *et al. (12)*. The sensory profile was analysed applying a one-way analysis of variance, with beer sample as a factor. Means, where required, were separated using Tukey's test.

Results and discussion

Physical–chemical characterization of beers

The results of the physical–chemical analyses are shown in Table 1. Real and apparent attenuation describes the degree of fermentation. Real attenuation (RA) is defined as:

$$RA = (OE - RE)/OE$$

where *OE*, original extract, represents the amount of extract before fermentation and *RE*, real extract, represents the amount of extract after fermentation *(20)* while the apparent attenuation (*AA*) is defined as:

$$AA = (OE - AE)/OE$$

where *AE*, apparent extract, represents the amount of extract after fermentation. This last parameter is influenced by the ethanol content *(21)*.

Table 1. Physical–chemical analysis of the wheat beers

Physical-chemical parameters	German	Czech	Italian
Original extract (°P)	12.59	11.71	13.10
Real extract (%)	4.46	5.14	5.75
Real attenuation (%)	64.60	56.10	56.10
Apparent extract (%)	2.53	3.56	4.00
Apparent attenuation (%)	79.90	69.60	69.50
Alcohol v/v (%)	5.37	4.34	4.90
Final attenuation (%)	80.2	80.0	77.5
Available extract (%)	2.49	2.34	2.96
pH	4.29	4.72	4.53
Total polyphenols (mg/L)	139	148	177
Colour (EBC units)	14.4	16.8	11.1
Bitterness (IBU)	10	17	22
Iso-α-acids (mg/L)	11.25	13.57	22.04
Titration acidity (1 M NaOH/100 mL)	3.10	2.28	3.48
Carbon dioxide (%)	0.74	0.63	0.49
Foam stability (s/3 cm)	214	194	261

As shown by the real and apparent attenuation values, fermentation progressed to a greater extent in the German beer and to a lesser extent in the two other beers, which showed similar values. Fermentation produces both ethanol and acids (such as citric and acetic acids), thus a high alcohol content is correlated with a decrease in the pH *(22)*. The beer with the highest alcohol content and lowest pH was the German wheat beer. However, the Sardinian wheat beer had higher values for the original and real extracts, thus containing more extract than the others. The ethanol content of beer is very important, from both economic and sensory points of view, as it is used to classify beers in terms of taxes and taste.

The total polyphenol content in beers was different, depending on the beer brand, with higher values being measured in the Italian wheat beer (Table 1). In the case of the Sardinian beer, a high polyphenol content was found as it contained more malted barley, whose polyphenol contribution to beer would be greater than that of wheat *(2)*. The Sardinian beer was made using 60% malted barley, while the other two beers were produced from 50% malted barley. Moreover, wheat proteins interact with polyphenols, forming insoluble precipitates during both fermentation and lagering. Therefore the increased presence of wheat proteins decreased the polyphenol content *(2)*. The colour of the Italian beer, expressed in EBC units, was less intense than that of the two other beers (Table 1). This value was within the range of values for wheat beers, as reported by Kunze *(23)*. Typically, the colour of the beer is due to malt and other raw materials that were used in the brewhouse *(22)* and is largely due to melanoidins and caramel present in the malt, although further caramelization can take place during wort boiling.

The Sardinian beer showed higher IBU values than the other two beers, and this is correlated with the higher *iso-α*-acid content of this beer. Acidity values and carbon dioxide content (%) were higher and lower respectively in the Sardinian beer. Foam stability of the Sardinian beer was greater than that of the German beer, probably owing to the fact that the Sardinian beer contained more protein since it was made from 20% durum wheat *(2)*.

Table 2. Esters and higher alcohol content of the wheat beers

Volatile compound	German (mg/L)	Czech (mg/L)	Italian (mg/L)
Ethyl formate	0.40	0.50	0.05
Ethyl acetate	22.60	25.80	12.20
Propyl acetate	0.01	0.01	0.03
2-Methylpropyl acetate	0.09	0.50	0.08
Ethyl butyrate	0.07	0.05	0.04
Butyl acetate	0.02	0.01	<0.01
3-Methyl-butyl acetate	2.00	3.80	1.20
Ethyl hexanoate	0.19	0.10	0.09
Ethyl-2-hydroxy propanoate	0.27	0.24	0.13
2-Phenyl-ethyl acetate	0.76	1.10	1.20
Ethylhexanol	0.50	0.47	0.46
Ethyl octanoate	0.24	0.16	0.28
Ethyl decanoate	0.02	0.05	0.11
Ethyl dodecanoate	<0.01	<0.01	0.92
Ethyl tetradecanoate	<0.01	<0.01	<0.01
Ethyl hexadecanoate	<0.01	<0.01	0.40
Total esters	27.17	32.79	17.19
n-Propanol	20.00	20.30	16.20
2-Methylpropanol	12.60	30.60	14.00
2- and 3-Methylbutanol	52.60	66.20	76.60
Furfurylalcohol	0.00004	0.00003	0.00012
2-Phenylethanol (FID)	14	9.6	18
Total alcohols	99.2	127.7	125.8

Volatile compounds of beers

The volatile components of the three beers are shown in Table 2. Esters, responsible for flavours described as floral, are formed by the esterification of fatty acids with ethyl alcohol and, to a lesser extent, with higher alcohols. The volatile components have high vapour pressures and are responsible for the aroma of beer. Their formation takes place during primary fermentation and the amount increases with an increasing duration of secondary fermentation.

Beer contains about 60 different esters and the values in Table 2 show that the Czech beer had the highest concentration of esters. This may be due to the conversion of ethanol into esters, as demonstrated by the fact that, although having equal degrees of fermentation, the Czech beer had a lower alcohol content than the Sardinian beer. The German beer had a greater degree of fermentation, and a higher alcohol content, ranking it second in volatile esters.

Higher alcohols are important organoleptic substances that give a fruity flavour to beer. They are formed by decarboxylation and reduction of α-keto acids derived as by-products in the metabolic processing of amino acids that are present in wort. They can also be formed during *de novo* biosynthesis of amino acids through carbohydrate metabolism, in which the corresponding α-keto acids are produced *(24)*. Data reported in Table 2 show that the Czech beer had a higher total alcohol content than the German beer, but was very similar to the Sardinian beer. This is probably due to a higher concentration of amino acids in the German wort. Concentrations of esters and alcohols vary depending on the type of beer, as reported by Pinho *et al.* (25). Wheat beers are characterized by a higher ester and alcohol content, resulting in a higher fruity flavour compared with barley beers.

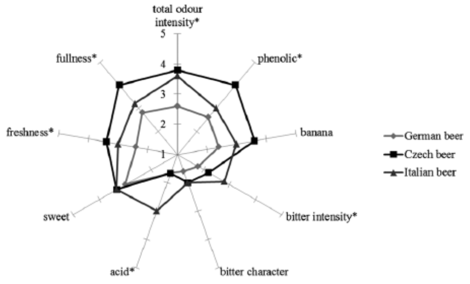

Figure 1. Wheat beer sensory profile. Asterisks indicate sensory attributes that are significantly different from the Tukey's test at the $p \leq 0.05$ level.

Sensory evaluation

In the sensory evaluations, the Sardinian wheat beer was deemed to be more balanced in taste with a more intense hoppy aroma, resulting in a beer that was less sweet than the other two beers (Fig. 1). This was probably due to the polyphenol content and especially to the high content of *iso*-α-acids that contribute most to beer bitterness. Acidity, which was higher in the Sardinian than the other two beers, contributed to the balanced taste of the Italian beer. As shown in Fig. 1, the Czech beer had a more fruity aroma and showed phenolic notes significantly different from the other two beers. The fruity character was probably due to the higher content of total esters, and in particular to 3-methylbutyl acetate, which confers a banana aroma to the beer (Table 2). This result was also confirmed by the total odour intensity attribute, which was significantly higher in the Czech beer compared with the others. The fullness, as represented by a sensation of heaviness in the mouth, and the freshness, causing a refreshing effect, was significantly higher in the Czech beer. The profile for the German beer, in almost all parameters evaluated, was less intense than the other two beers. The sensory profile therefore correlated with the physical–chemical parameters of the beers and especially with the volatile components.

Conclusions

In this study, a Sardinian durum wheat beer was compared with two other standard wheat beers from different countries. The results of the real degree of fermentation were higher for the German beer, showing that fermentation occurred to a greater extent than for the other beers, and this was confirmed by the highest alcohol content and lowest pH of the German beer. However, the Sardinian beer had the highest values for original and real extracts, together with the highest polyphenol content. The Czech beer had the highest concentration of volatile esters and total alcohols. In sensory evaluations, the Sardinian beer was deemed to be more balanced in taste, and less sweet. This is the first comparative characterization of an Italian wheat beer, and in particular of a Sardinian craft durum wheat beer. More research will be required to develop correlations between sensory and volatile profiles with additional wheat beers, but this preliminary report offers some insights into Sardinian durum wheat beer.

Acknowledgements

Ilaria Mascia gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (POR Sardegna FSE Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.). The authors thank the Ministry of Agriculture of the Czech Republic (project QI111B053) for its financial support, and they wish to express their sincere thanks to Lara micro-brewery for the beers.

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5 CHAPTER II

Is it possible to create an innovative craft durum wheat beer with sourdough yeasts? A case study

Journal of the Institute of Brewing

Is it possible to create an innovative wheat durum craft beer with sourdough yeasts? A case study

Journal:	*Journal of the Institute of Brewing*
Manuscript ID:	Draft
Wiley - Manuscript type:	Research article
Date Submitted by the Author:	n/a
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Keywords:	sourdough, craft beer, micro-brewery, volatile compounds
Abstract:	Craft beer is typically defined as a non-filtered, unpasteurized and bottle re-fermented beer, whose production is aimed at maintaining its original sensory characteristics. The intent of most micro-breweries is to meet these requirements in producing a high quality beer. In Italy, as well as in Sardinia, the number of micro-breweries is increasing. In an attempt to make this product more original, new strains of Saccharomyces cerevisiae were selected. Sourdough yeasts were used as an alternative source of starters for beer production, since they are able to ferment maltose. In this work two wheat beers were compared, one brewed with an autochthonous yeast isolated from sourdough and the other brewed with a commercial yeast normally used in micro-breweries. The wheat beer fermented with the autochthonous yeast had the highest ethanol content, the lowest pH and the highest content of esters and alcohols. From the sensory point of view the two beers showed significant differences in the triangle test but were not significantly different in the paired test with preference.

Is it possible to create an innovative wheat durum craft beer with sourdough yeasts? A case study

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Abstract

Craft beer is typically defined as a non-filtered, unpasteurized and bottle re-fermented beer, whose production is aimed at maintaining its original sensory characteristics. The intent of most micro-breweries is to meet these requirements in producing a high quality beer. In Italy, as well as in Sardinia, the number of micro-breweries is increasing. In an attempt to make this product more original, new strains of *Saccharomyces cerevisiae* were selected. Sourdough yeasts were used as an alternative source of starters for beer production, since they are able to ferment maltose. In this work two wheat beers were compared, one brewed with an autochthonous yeast isolated from sourdough and the other brewed with a commercial yeast normally used in micro-breweries. The wheat beer fermented with the autochthonous yeast had the highest ethanol content, the lowest pH and the highest content of esters and alcohols. From the sensory point of view the two beers showed significant differences in the triangle test but were not significantly different in the paired test with preference.

Keywords: sourdough, craft beer, micro-brewery, volatile compounds

Introduction

Fermentation is a common factor in the production of all alcoholic beverages. In beer, yeast converts sugars, primarily derived from malt, into ethanol. Not only are the yeast strains important in determining the nature of any alcoholic beverage, but even the substrate, i.e. the raw material on which the yeast acts, has a role in determining the properties of the final product. The character of beer is therefore achieved by the interaction of selected strains of yeast with the wort, which is formed from malt and hops (1). *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* are two fermentation yeasts that are usually used in micro-breweries as starter cultures (2). The choice of the yeast to inoculate during fermentation is important in brewing regardless of the type of beer being produced, whether it be a craft or an industrial beer. Beers produced with different yeasts may have different characteristics, affecting particularly the aromatic composition of beers (3).

Micro-breweries in Italy are always in a state of continuous expansion. In 2007, 175 micro-breweries were operating, and this number increased to greater than 500 by 2010. Today, craft beers in Italy represent 4-5% of the total beer production (3). In Sardinia, the number of micro-breweries is also increasing, and today we estimate the presence of 23 active micro-breweries (4).

The term "craft beer" typically refers to a beer that is not pasteurized, is un-filtered and is re-fermented in the bottle (3, 5), and whose main attribute is that original sensory characteristics are maintained. The term micro-brewery is used when referring to small independent breweries that produce a limited amount of beer. The beers produced in micro-breweries are distinguished by originality of taste and flavour, obtained using traditional methods that give a certain quality to the finished product.

To produce a more original craft beer, new starters for improving quality of beer have to be selected (6, 7).

The selection of new strains for craft beers from the hopped wort is a challenge due to the low viability of wild yeasts. Moreover, the widespread use of commercial starter strains makes the selection of wild yeast quite difficult (5).

Studies on the selection of new starters have been made on *S. cerevisiae* strains isolated from other food matrices. The general criterion that was used by the beer industry for strain selection was their capability to rapidly and completely ferment wort-fermentable sugars (8).

The sourdoughs, which are intermediate fermented products used in the production of bread and baked goods (9), have been hypothesized to be an alternative source of starters for beer production. *S. cerevisiae* is present in the sourdoughs, together with other yeasts (10). A recent study on sourdoughs shows that these strains are able to ferment maltose, the main carbohydrate in beer (11). In fact these strains also showed a very good uptake and ability to utilize trehalose, which shares common transporters with maltose (11).

For this reason, the aim of this study was to evaluate the possibility to produce an innovative durum wheat beer fermented by a sourdough strain of *S. cerevisiae*. As reference, the same beer was produced by using a commercial yeast. An "original" durum wheat beer will add an element of originality to existing production, thus creating a novel link with the territory.

Material and methods

Sardinian wheat beers

Two craft durum wheat beers were brewed, in duplicate, in Sardinia; one with commercial yeast (CY) and the other with an autochthonous yeast (AY). The commercial yeast was the Safbrew S33 strain (Fermentis, Lesaffre Italia S.P.A., Italy), normally used by brewers for top-fermented wheat beers, whereas the autochthonous yeast *S. cerevisiae* S42 isolated from a craft Sardinian sourdough, belongs to the yeasts collection of the Department of Agraria (University of Sassari). The two beers were produced with 60% of malted barley, 20% of commercial malted wheat, and 20% of "Cappelli wheat", an old variety of unmalted durum wheat that grows in Sardinia, following the same technological protocol: mashing (infusion process) as reported in Mascia et al. (12); after mashing the temperature was decreased to 20°C and the yeast strains were inoculated ($1x10^6$ cells/ml); primary fermentation was conducted in the vessel in static conditions at a temperature of 20°C for 2 days. Then, the temperature was decreased to 18°C and kept for three days. Finally, the temperature was raised to 20°C and maintained for three days. Then, the second fermentation (maturation) was carried out in the bottle at 18-20°C for 7-10 days, after which the temperature was decreased to 10°C and kept for other 7 days. The bottles were then stored in a cold room at a temperature of 4°C. No pasteurization or filtration was carried out. The beers were analyzed within two months.

Reagents

Ethyl acetate, ethylbutyrate, 1-propanol, isobutanol, iso-amyl acetate, ethylcaproate, isopentanol, ethylcaprylate, acetic acid, 2-ethyl-1-hexanol, linalool, ethyl caprate, 2-phenylethanol, ethylmyristate, caprylic acid, ethyl palmitate, capric acid. Heptanoic acid ethyl ester Internal Standard, 3-octanol Internal Standard were of purity > 99% and were obtained from Sigma-Aldrich (USA). Helium 6.0 quality gas (Messer, Czech Republic) was used for GC/MS analysis.

Chemical-physical analyses

Beer samples were analyzed through the Alcolyzer (Anton Paar GmbH, Austria) for apparent extract, real extract, alcohol content, original extract, apparent attenuation and real attenuation.
Determinations of bitterness, total polyphenols and pH were made in accordance with EBC methods 9.8, 9.11 and 9.35 (13).

Volatile analysis

Volatile compositions were quantified by headspace-solid phase micro extraction (HS-SPME) coupled with gas chromatography (GC – Agilent 6890N) using a mass spectrometry detector (MSD – Agilent 5975B Inert) according to the method of Pinho et al. (14). Extraction and concentration of the volatile compounds was carried out using the HS-SPME technique with a 75 µm carboxen/polydimethylsiloxane (CAR–PDMS) fiber (Supelco, Bellefonte, PA, USA), and 30 m x 0.25 mm x 0.25 µm HP-INNOWax Polyethylene Glycol column (Agilent Technologies, Santa Clara, CA, USA). Helium 6.0 was the carrier gas at a flow rate of 1.1 mL/min. The injection temperature was 260°C. The oven temperature program was the following: 10 min at 30°C, followed by an increase of 2°C/min to 52°C for 2 min, an increase again of 2°C/min to 65°C , and of 5°C/min to 250°C for 3 min.
An amount of NaCl (2 g) was added in order to increase the volatility of compounds in the HS. Each analysis was carried out in duplicate. Peak areas were normalized using heptanoic acid ethyl ester as an internal standard for esters and acids, and 3-octanol as an internal standard for alcohols. The concentrations of volatiles were determined using external calibration.

Sensory analysis

An overall difference test, the triangle test, was performed to determine if a difference existed between the CY and AY beers. A panel of thirty untrained assessors was asked to determine the odd sample (15) among three samples, where two were the same and the other was different. Beer samples were presented to the assessors in a balanced and randomized order (16). The triangle test was performed twice on duplicate beer samples. Then, to identify if one of the two beers, CY and AY, was preferred, a paired preference test was performed with a panel of eighty consumers (17).

Statistical analysis

Results of physical-chemical analyses and volatile compositions were analyzed with the *t*-test ($p< 0.05$), using Statistica 6.0 for Windows. The triangle test was analyzed using the critical values table for triangle test, while the paired preference test was analyzed using the critical values table for paired comparison test for difference (18).

Results and discussion

Comparison of wheat beers by physical-chemical analysis

The data shown in Table 1 represent physical-chemical analyses that were carried out in duplicate on CY and AY beer samples. The values of real and apparent attenuation show that fermentation progressed to a greater extent in the AY beer. This was confirmed by the highest value for alcohol content and by the lowest value for pH of the AY beer. Indeed, in the fermentation process, ethanol and acids (such as citric and acetic acids) are produced, increasing the alcohol content and lowering the pH (19). Probably due to this reason, the AY beer has a higher content of volatile substances compared with the CY beer. The polyphenolic content was higher in the CY beer. A possible explanation is that during fermentation and lagering of the beer brewed with autochthonous yeast, more proteins could have interacted with polyphenols, forming insoluble precipitates, as reported in Depraetere et al (20). As expected, due to the same hop content, bitterness was not significantly different between the two beers.

Comparison of volatile compounds in wheat beers

The results of GC-MS analysis of volatile compounds in CY and AY beers are shown in Table 2. In general, the AY beer had a level of volatile substances that was higher than CY beer. This could be due to a greater degree of fermentation, as evidenced by higher values for real and apparent attenuation, alcohol content and by the lower pH.

Volatile alcohols and esters are responsible for the fruity character of beer. Esters are produced by yeast cells during the fermentation process, catalyzed by intracellular enzymes. The results in Table 2 show that the AY beer had the highest concentration of esters. This may be due to a much greater conversion of ethanol into esters, as demonstrated by the higher degree of fermentation, and by the higher alcohol content of the AY beer. In addition, it can be seen that the AY beer had an acid concentration that was higher than that of the CY beer. A possible reason is related to the strain of yeast and to the degree of fermentation of the AY beer, being higher than CY. In fact, as reported in Horák et al. (21), the production of acids is due to factors such as original wort gravity, wort composition, aeration, temperature, and also to the yeast strain being used. Data reported in Table 2 show that the AY beer had a high concentration of alcohol. The alcohols can be generated from α-keto acids that are formed during *de novo* biosynthesis of amino acids through carbohydrate metabolism or during the catabolism of amino acids by the Ehrlich pathway (22). A possible explanation for the difference between AY and CY beers can be that the higher fermentation rates of AY resulted in a high request of assimilable nitrogen for yeast metabolism and growth. This request could have been satisfied with the catabolism of amino acids through the Ehrlich pathway (22).

Sensory evaluation of CY and AY wheat beers

Results obtained from the triangle test showed that the untrained assessors found a significant difference between the CY and AY beers at a p level < 0.05. The test was performed twice and the results were consistent. Results of the consumer test, which was a paired preference test, revealed no significant difference between the CY and AY beers at a p level < 0.05. Again, a tendency to prefer the AY beer was indicated, probably due to the greater fruity character of the AY beer, even though this tendency was not significant.

Conclusion

Two Sardinian craft durum wheat beers, one fermented with a commercial yeast and the other with a sourdough yeast, were compared. The values of real and apparent attenuation show that fermentation progressed to a greater extent in the AY beer. This was confirmed by the higher alcohol content and by the lower pH in the AY beer. Probably for this reason, the AY beer had a higher content of volatile substances compared with the CY beer. The results of the triangle test showed a significant overall difference between the two beers, while the paired preference test did not show any significant difference in preference between them, even though a tendency to prefer the AY beer was observed.

The use of durum wheat and AY could provide an additional element of originality to the existing production, thus creating a further link with the territory that could have major repercussions from an economic point of view. In fact this is the first time that a durum wheat beer made using AY isolated from sourdoughs has been produced. These interesting results could be the basis for enhanced originality of Sardinian "original" durum wheat beer and could lead to the expansion of export markets outside of the region.

Acknowledgements

Ilaria Mascia gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (POR Sardegna FSE Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.). The authors thank the Ministry of Agriculture of the Czech Republic (project QI111B053) for its financial support, and the Italian MIPAAF (Ministero delle Politiche Agricole Alimentari e Forestali) for granting the PROBIRRA project (Artisanal beer production with barley, hop and yeasts produced and isolated in Sardinia) under the OIGA 22805 announcement 10/15/2010 D.M. n. 22299 10/20/2011. The authors wish to express their sincere thanks to Lara micro-brewery for the technical help provided during the project.

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Table 1. Comparison of AY and CY wheat beers by physical-chemical analyses

	Apparent extract (%)	Real extract (%)	Alcohol v/v (%)	Original extract (Plato)	Apparent attenuation (%)	Real attenuation (%)	pH	Bitterness (IBU)	Total polyphenols (mg/L)
AY	3.70^{b}	5.49^{b}	4.99^{a}	12.99^{a}	71.5^{a}	57.7^{a}	4.30^{b}	23^{a}	169^{b}
CY	4.10^{a}	5.81^{a}	4.75^{b}	12.93^{a}	68.4^{b}	55.1^{b}	4.81^{a}	23^{a}	185^{a}

Values with different letters are significantly different at $p< 0.05$ by Student *t*-test

Table 2 GC-MS measurement of volatile compounds of AY and CY wheat beers

Volatile compound	AY (mg/L)	CY (mg/L)
Ethyl acetate	15.88[a]	12.40[b]
Ethyl butyrate	0.02[a]	0.01[a]
Isoamyl acetate	1.24[a]	0.86[b]
Ethyl caproate	0.03[a]	0.02[a]
Ethyl caprylate	0.31[a]	0.29[a]
Ethyl caprate	0.11[b]	0.19[a]
Ethyl myristate	0.03[a]	0.05[a]
Ethyl palmitate	0.03[a]	0.05[a]
Acetic acid	6.83[a]	6.41[b]
Caprylic acid	4.35[a]	3.52[b]
Capric acid	0.85[b]	0.98[a]
1-Propanol	5.25[b]	9.60[a]
Isobutanol	17.33[a]	7.39[b]
Isopentanol	21.71[a]	11.63[b]
2-Ethyl-1-hexanol	0.10[a]	0.12[a]
Linalool	1.65[a]	1.78[a]
2-Phenylethanol	15.10[b]	15.47[a]

Values with different letters are significantly different at $p< 0.05$ by Student *t*-test

6 CHAPTER III

Assessment of craft durum wheat beer during aging

Elsevier Editorial System(tm) for Food Chemistry
Manuscript Draft

Manuscript Number:

Title: Assessment of craft durum wheat beer during aging

Article Type: Research Article (max 7,500 words)

Keywords: shelf life; craft beer; carbonyl compounds; flavour stability

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Abstract: The shelf life of three different craft durum wheat beers, brewed in a Sardinian micro-brewery, fermented with autochthonous yeasts isolated from sourdough and commercial yeasts, were studied over a period of six months at two different temperatures: shelf temperature (28°C) and normal cold storage temperature (8°C). The results showed that the three beers had similar physico-chemical, volatile and sensory characteristics. This suggests the possible use of autochthonous yeasts in the brewing process to create a stronger link with the local region. During aging, a decrease in the content of esters and higher alcohols, and an increase in carbonyl compounds occurred at the higher temperature. No significant differences were found in physico-chemical parameters during storage except for the color, which increased at 28°C. Six months of aging did not significantly modify sensory perception of the beers.

Assessment of craft durum wheat beer during aging

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Abstract

The shelf life of three different craft durum wheat beers, brewed in a Sardinian micro-brewery, fermented with autochthonous yeasts isolated from sourdough and commercial yeasts, were studied over a period of six months at two different temperatures: shelf temperature (28°C) and normal cold storage temperature (8°C). The results showed that the three beers had similar physico-chemical, volatile and sensory characteristics. This suggests the possible use of autochthonous yeasts in the brewing process to create a stronger link with the local region.

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Keywords: shelf life; craft beer; carbonyl compounds; flavour stability

Chemical compounds studied in this article

1-Propanol (PubMed CID: 1031); Isobutanol (PubMed CID: 6560); Iso-amyl acetate (PubMed CID: 31276); Ethylcaproate (PubMed CID: 102916); Isopentanol (PubMed CID: 31260); Ethylcaprylate (PubMed CID: 7799); 2-Ethyl-1-hexanol (PubMed CID: 7720); Ethyl caprate (PubMed CID: 8048); 2-Phenylethanol (PubMed CID: 6054); Ethyl myristate (PubMed CID: 31283)

1. Introduction

Beer aging is one of the most important factors that influences the characteristics of beers and it is usually considered a quality problem. During storage, aging compounds are formed in bottled beers, causing the development of aging flavors and changes in taste considered to be unpleasant by consumers. The consumer recognizes a brand of beer, associating it with a certain flavor characteristic of that beer. It is therefore essential for brewers to maintain the characteristic flavor of their product and to produce a beer of constant quality over time (Stephenson & Bamforth, 2002; Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006). However, the formation of aged flavors cannot be controlled, and it is difficult for brewers to ensure repeatability and stability of beer flavor over time.

From the chemical point of view, the phenomenon of aging has therefore been studied for many years (Bamforth, 1999; Hashimoto & Kuroiwa, 1975; Kaneda, Kobayashi, Takashio, Tamaki, & Shinotsuka, 1999).

The complexities of malt production and beer composition are the reasons why many factors modify flavor stability in the final product (Bamforth, 2004). During storage, a change in the chemical composition of beer occurs. The principal aging substances that are formed are carbonyl compounds, in particularly aldehydes (Soares da Costa, Gonçalves, Ferreira, Ibsen, Guedes de Pinho, & Silva Ferreira, 2004; Dong, Piao, Zhang, Zhao, Hou, & Shi, 2013). These can be produced by different reactions such as oxidation of higher alcohols, degradation of bitter acids, Strecker degradation of amino acids, oxidation of unsaturated fatty acids, and aldol condensations (Bamforth, 1999; Bamforth, 2004; Vanderhaegen et al., 2006; Wackerbauer & Hardt, 1997). Regarding sensory attributes, the presence of aging compounds generally leads to the development of sweet tastes and a cardboard flavor (Dalgliesh, 1977; Narziss, 1986). During storage, a decline in beer bitterness was also confirmed and could become a marker for estimations of oxidative damage (Karabin et al., 2014).

"Craft beer" refers to a beer that is not pasteurized, un-filtered, and is re-fermented in the bottle (Bokulich & Bamforth, 2013; Canonico, Comitini, & Ciani, 2014). The great importance of these beers lies in their original sensory features and distinctive elements of taste and flavor that differentiate them from industrial beers. Craft beers are produced by micro-breweries and, usually are destined for the local market. Recently, the rise in globalization has led to the need for broadening of the market and the export of craft beers beyond their local territories. For this reason, transport and storage time have increased, and as a consequence, the need to increase craft beer shelf life. Most studies on beer shelf life have focused only on industrial lager beers, since these are the most prevalent beers on the market (Vanderhaegen et al., 2006). To our knowledge, no studies reported in the literature have focused on craft beer shelf life, particularly craft wheat beers.

In this work we studied the physico-chemical characteristics, sensory profiles and the evolution of volatile compounds during storage of three different craft durum wheat beers, over a period of six months at two different temperatures: shelf temperature (28°C) and normal cold storage temperature (8°C). The three beers were brewed in a microbrewery located in the south-east of Sardinia, Italy, and differ only in the yeast that was used for brewing, one commercial and two autochthonous. Here, we present, for the first time, an assessment of craft wheat beers during aging. The main intent was to investigate the possibility of expanding the market for craft beers outside of the region.

2. Material and methods

2.1 Sardinian wheat beers and aging conditions

Three craft durum wheat beers were brewed in duplicate, in Sardinia with three different yeasts, one commercial and two autochthonous. The commercial yeast was the Safbrew S33 strain (Fermentis, Lesaffre Italia S.P.A., Italy), normally used by brewers to produce top-fermented wheat beer, whereas the autochthonous yeasts *S. cerevisiae* S42 and S38 were isolated from a craft Sardinian sourdough by the Microbiology section of the Department of Agraria (University of Sassari), and selected for their capacity to ferment maltose (Marongiu et al., 2014). The three beers were produced with 60% of malted barley, 20% of commercial malted wheat, and 20% of "Cappelli" wheat, an old variety of unmalted durum wheat that grows in Sardinia, following the technological protocol reported by Mascia, Fadda, Dostálek, Olšovská, and Del Caro (2014). Aging conditions of the beers were as follows: after their production, half of the beers were kept at a temperature of 8°C (L = Low) in a refrigerated chamber, representing normal cold storage for beer, and the other half at a temperature of 28°C (H = High), in a dark room at a controlled temperature, representing shelf temperature. The beers, stored at the two temperatures, were first analyzed after two months, which is the storage period chosen by this microbrewery before marketing (time 0 = t0) and then every 40 days from t0, over a total period of 6 months (time 1 = t1 and time 2 = t2).

2.2 Chemicals

The following chemicals, with purity > 99%, were supplied by Sigma-Aldrich (USA): ethyl acetate, ethylbutyrate, 1-propanol, isobutanol, iso-amyl acetate, ethylcaproate, isopentanol, ethylcaprylate, acetic acid, 2-ethyl-1-hexanol, linalool, ethyl caprate, 2-phenylethanol, ethyl myristate, caprylic acid, ethyl palmitate, capric acid, heptanoic acid, ethyl ester, 3-octanol, 2-methylpropanal, 3-methylbutanal, penta-2,3-dione, furfural, heptanal, benzaldehyde, (E)-2-nonenal, diacetyl, 3-fluorobenzaldehyde, and O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBOA).

2.3 Physico-chemical analysis

Beer samples were analyzed through the Alcolyzer (Anton Paar GmbH, Austria) for apparent extract, original extract, and alcohol content. Standard beer analyses and determination of colour, bitterness and pH were carried out according to EBC methods 9.6, 9.8, 9.35 (EBC, 2010).

2.4 GC-MS of alcohols and esters

Volatile compounds were quantified by headspace-solid phase micro extraction (HS-SPME) coupled with gas chromatography (GC – Agilent 6890N) and a mass spectrometry detector (MSD – Agilent 5975B Inert) according to the method of Pinho, Ferreira, and Santos (2006). Extraction and concentration of the volatile compounds was carried out using HS-SPME with a 75 µm carboxen/polydimethylsiloxane (CAR–PDMS) fiber (Supelco, Bellefonte, PA, USA), and 30 m x 0.25 mm x 0.25 µm HP-INNOWax polyethylene glycol column (Agilent Technologies, Santa Clara, CA, USA). Helium 6.0 was the carrier gas, at a flow rate of 1.1 mL/min, and the injection temperature was 260°C. The following oven temperature program was used: 10 min at 30°C, followed by an increase of 2°C/min to 52°C for 2 min, an increase again of 2°C/min to 65°C, and of 5°C/min to 250°C for 3 min.

An amount of NaCl (2 g) was added in order to increase the volatility of compounds in the HS. All analyses were carried out in duplicate. Peak areas were normalized using heptanoic acid ethyl ester as an internal standard for esters and acids, and 3-octanol as an internal standard for alcohols. The concentrations of volatiles were determined using external calibration.

2.5 Carbonyl compounds

Carbonyl compounds were identified and quantified in duplicate by HS-SPME coupled with gas chromatography mass spectrometry (GC-MS) according to the method of Moreira et al., based on SPME with on-fibre derivatization (Moreira, Meireles, Brandão, & de Pinho, 2013). The extraction and concentration of carbonyl compounds was carried out using HS-SPME with 50/30µm divinylbenzene/carboxen/polydimethylsiloxane (DVB–CAR–PDMS) fiber (Supelco, Bellefonte, PA, USA). 200 µL of PFBOA solution and 10 mL of deionized water were placed in a 20 mL glass vial. The DVB–CAR–PDMS fiber was then placed in the headspace of the PFBOA solution for 20 min a 50°C to allow for derivatization. The fiber loaded with PFBOA was then exposed to the headspace of 10 mL of beer and 100 µL of 3-fluorobenzaldehyde (IS) placed in a 15 mL vial. Fibre desorption time was 600 s. The column used was a 30 × 0.25 mm × 0.25 µm HP-5 MS (Agilent Technologies, Santa Clara, CA, USA). Helium 6.0 was the carrier gas at a flow rate of 1.1 mL/min, and the injection temperature was 250°C. The temperature program was the following: 2 min at 40°C, ramped at a rate of 10°C/min to 140°C and of 7°C/min to 250°C for 14 min. The concentrations of carbonyl compounds were determined using external calibration.

2.6 Sensory analysis

Descriptive sensory analysis was performed on the three durum wheat beers (fermented with S33, S42 and S38 yeasts) using a panel of 12 assessors, selected and trained following the ASBC methodology (American Society of Brewing Chemists, 2014).

All the beers were analyzed in duplicate after three different storage times (t0, t1 and t2) for a period of 6 months at two different temperatures, L and H. Three sessions for each storage time were performed: every session assessor evaluated four beers served in a randomized and balanced order (Mac Fie, Bratchell, Greenhoff, & Vallis, 1989) at serving temperature (usually 8°C). Beers stored at 28°C were put in the refrigerator the day before tasting to equilibrate the temperature to 8°C.

Panel performance was evaluated on two samples replicated three times using PanelCheck V1.4.0 software (Nofima Mat, Ås, Norway). The attributes chosen to describe the products were the following: color and persistency of the foam, fruity, malt and yeast character for odor, bitter and acid for taste and freshness and fullness for mouth-feel sensations. Four off-flavours, oxidized, cardboard, sweetcorn and phenolic, were selected to evaluate and quantify their presence during aging. Assessors evaluated all attributes on a 10 cm unstructured scale (0 = absence or low intensity and 10 = maximum intensity). Assessments were calibrated using reference standards for the chosen attributes, for at least two points of the scale.

2.7 Statistical analysis

Principal Component Analysis (PCA) was performed using the R-based software developed by the Chemometrics group of the Division of Analytical Chemistry of the Italian Chemical Society (http://gruppochemiometria.it/).

A Three-way ANOVA, with the factors yeast, storage time and temperature, and their interactions, was used to identify significant differences among the samples in the volatile, chemical, and sensory profiles. Significant means were separated using the LSD Fisher's test ($p<0.05$). The software used was Statistica 6.0 for Windows.

3. Results and discussion

The three craft durum wheat beers, one brewed with commercial yeast (S33) and two with autochthonous yeasts (S42, S38) isolated from sourdough, were compared for their aging characteristics. PCA analyses of the three beers, carried out on all the physico-chemical and sensory variables, at three different storage times (t0, t1 and t2) and at two temperatures (L and H) are shown in Fig. 1.

Examining the score plot (Fig. 1a), the influence of storage time is clear, with a distinction between t0 and t1 and between t0 and t2, with a less marked distinction between t1 and t2. Separation between t0 and the other two storage times is on PC1 while that between t1 and t2 occurs along both the first and the second components. It is clear that variability due to storage time was much greater than that due to different temperatures. Moreover, the temperature effect was not clear, with a separation of samples at L and at H temperatures being less evident for t1 than for t2.

At t0, several variables. including acetic, capric and caprylic acids, 1-propanol, and linalool, ethyl myristate, caprylate, and caprate and beer color showed higher values. These variables decreased in concentration after t1 and a further decrease after t2. Differences between t0 and t1 were larger than those between t1 and t2. Concentrations of chemicals such as 2-methylpropanal, isoamylacetate and ethyl acetate decreased over time, in particular at t2. Concentrations of ketones and aldehydes such as heptanal, furfural, (E)-2-nonenal, diacetyl and pentan-2,3-dione were low at t0, as expected, but increased during storage at the two temperatures (Fig. 1b). In fact, moving from t1 to t2, heptanal, furfural, diacetyl, penta-2,3-dione and (E)-2-nonenal increased, but the effect was greater when the temperature was 28°C.

In general, a decrease in esters and alcohols and an increase in carbonyl compounds occurred during aging, as reported in several papers (Vanderhaegen, Neven, Coghe, Verstrepen, Verachtert, & Derdelinckx, 2003; Malfliet et al., 2008). Our results confirmed data reported in the literature. It is clear that storage temperature played a role in the decrease in volatile components but to a lesser extent than storage time. In fact, for the two temperatures, the decrease was more evident at t2 (Fig. 1b). The decreased concentration of esters during aging depends on the presence of yeast-derived extracellular esterases that catalyze ester breakdown (Neven, Delvaux, & Derdelinckx,1997).

Carbonyl compounds that are formed during aging are responsible for the stale flavor in beer. They are formed as products of oxidative and non-oxidative reactions. In particular, 3-methylbutanal and 2-methylbutanal may be derived from Strecker degradation of the amino acids leucine, isoleucine and phenylalanine, while vicinal diketones such as diacetyl and penta-2,3-dione, are synthesized by Maillard reactions (Vanderhaegen et al., 2003).

(E)-2-Nonenal is the carbonyl compound responsible for the cardboard flavor characteristic of aged beer. It originates from the degradation of linoleic acid and is often used as a key indicator for beer preservation. Improved brewing technologies may decrease its concentration by limiting lipid oxidation. In the beers analyzed here, this compound tended to increase during storage time at the two temperatures (Fig. 1b).

For the sensory attributes, at t0, fruity odour, malt odour, fullness, acid, freshness and bitter were more intense, then decreased during storage. The sensory attributes acid, bitter, malt aroma, sweetcorn and fullness decreased between t1 and t2. Attributes such as cardboard, phenolic, oxidized and sweetcorn were present in the beers during storage although their perception, even at the end of storage, was only approximately 1 on a scale of intensity from 0 to 10.

Some sensory attributes were positively or negatively correlated with chemical compounds: sweetcorn, cardboard, phenolic and oxidized were highly positively correlated with each other, and with the carbonyl compounds penta-2,3-dione, diacetyl, 2-methyl and 3-methyl butanal (Vanderhaegen et al., 2003) as expected, while freshness, fruity and fullness are positively correlated with 2-methylpropanal and acetic acid (Moreira et al., 2013).

The sourdough yeast S38 differed from the other two yeasts, sourdough yeast S42 and the commercial yeast S33, with a more fruity odor and a higher content of alcohols and esters such as isoamylacetate and ethyl acetate.

Differences between the two storage temperatures (L and H) were clearly shown in the three-way ANOVA results (Table 1), where a significant reduction in esters and alcohols, and a significant increase in carbonyl compounds, were shown at the highest temperature. Among the physico-chemical parameters, only color was significantly different between the two temperatures, with an increase at the higher temperature. This was due to the formation of Maillard reaction and polyphenol oxidation products (Vanderhaegen, Delvaux, Daene, Verachtert, & Delvaux, 2007). No significant differences in sensory profile were found for the two temperatures.

The sensory profiles for the three durum wheat beers fermented with the three yeasts at t0 are shown in Fig. 2.

No significant differences were found at t0 among the beers for all the sensory attributes except fullness, which was higher in S42 beer (autochthonous yeast) compared with S33 beer (commercial yeast) andS38 beer (autochthonous yeast). There was a tendency for yeast S38 to be more fruity that the others, but this difference was not significant.

Sensory profiles of beers fermented with the three yeasts, for t1 and t2 storage times at two different temperatures are shown in Fig. 3, 4 and 5.

Sensory profiles for each yeast highlight the influence of aging on sensory characteristics but not an effect of storage temperature, as reported previously. Generally speaking, the intensity of sensory attributes at t1 were higher than at t2. This result is more evident in the S33 and S38 profiles (Fig. 3 and Fig. 5) where characteristics such as foam color (for both beers), yeast odor (for S33 beer), fruity odor (for S38 beer), freshness (for both beers) and fullness (for S38 beer) were significantly different ($p < 0.05$). Only the off-flavor, sweetcorn, was significantly different in S33 beer, although the intensity was less than 2 on a scale of 1 to 10. No significant differences were found in the S42 profile (Fig. 4) during aging.

4. Conclusion

Three Sardinian craft durum wheat beers, produced in a micro-brewery located in the south-east of Sardinia, were assessed during aging to evaluate flavor stability with variations in yeasts, time, and temperature.

Volatile components, analyzed by GC-MS, changed over a period of six months with a decrease in esters and higher alcohols and an increase in carbonyl compounds. This trend was more evident at the higher temperature.

In terms of physico-chemical parameters, no significant differences were found regarding time, temperature or yeasts; only color was significantly different between the two temperatures, increasing at the higher temperature.

The sensory profiles showed a reduction in some attributes related to flavor and in-mouth sensations during storage, confirming data obtained by GC-MS, but no significant differences were reported by the panel for intensity of off-flavors, even if the GC-MS analysis showed an increase in carbonyl compounds. No significant differences were observed between storage temperatures. Differences between yeasts at t0 were found only for fullness, being more intense in one of the autochthonous yeasts compared with the commercial one.

In conclusion, the three beers, brewed with different yeasts, had similar physico-chemical, volatile and sensory characteristics during aging. This suggests the possible use of autochthonous sourdough yeasts in brewing, providing an additional element of authenticity to existing production in order to create a stronger regional link, with positive economic returns.

We have presented here, the first assessment of behavior during aging of craft durum wheat beers. Future studies will focus on shelf life of beers over a longer period in order to improve product stability and thus enhance the potential for expanding the craft beer market outside of the local region.

Acknowledgements

Ilaria Mascia gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (POR Sardegna FSE Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.). The authors thank the Ministry of Agriculture of the Czech Republic (project QI111B053) for its financial support, and they wish to express their sincere thanks to Lara micro-brewery for the technical help provided during the project. The authors thank Professor J. D. Brooker for editing the manuscript.

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Figures legend

Fig. 1. PCA score (a) and loading plots (b) of the physico-chemical and sensory attributes of the beer samples studied at t0 (blue), t1 (purple), and t2 (red) and at L and H temperatures.

Fig. 2. Sensory profiles of the three durum wheat beers at t0.

Fig. 3. Sensory profiles of durum wheat beer fermented with S33 (commercial yeast) during storage for two times at two temperatures

Fig. 4. Sensory profiles of durum wheat beer fermented with S42 (autochthonous sourdough yeast) during storage for two times at two temperatures

Fig. 5. Sensory profiles of durum wheat beer fermented with S38 (autochthonous sourdough yeast) during storage for two times at two temperatures

Table 1

Table 1 Influence of the storage temperature on volatiles and physico-chemical profiles of craft durum wheat beers.

	Low	High
Esters (mg/L)		
Ethyl acetate	8.53[a]	6.69[b]
Ethyl caprylate	1.37[a]	0.92[b]
Ethyl caprate	1.42[a]	0.71[b]
Ethyl myristate	0.22[a]	0.15[b]
Alcohols (mg/L)		
1-Propanol	0.02[a]	0.01[b]
Linalool	0.63[a]	0.54[b]
Carbonyl compounds (µg/L)		
2-Methylpropanal	6.25[b]	7.14[a]
2-Methylbutanal	15.84[b]	18.56[a]
3-Methylbutanal	85.07[b]	102.35[a]
Penta-2,3-dione	15.71[b]	18.14[a]
Furfural	0.27[b]	0.30[a]
Heptanal	4.87[b]	5.59[a]
(E)-2-Nonenal	1.39[b]	2.05[a]
Diacetyl	0.44[b]	0.49[a]
Physico-chemical compound		
Colour (EBC units)	6.09[b]	6.36[a]

Values with different letters are significantly different at $p<0.05$ by LSD Fisher test

Fig. 1
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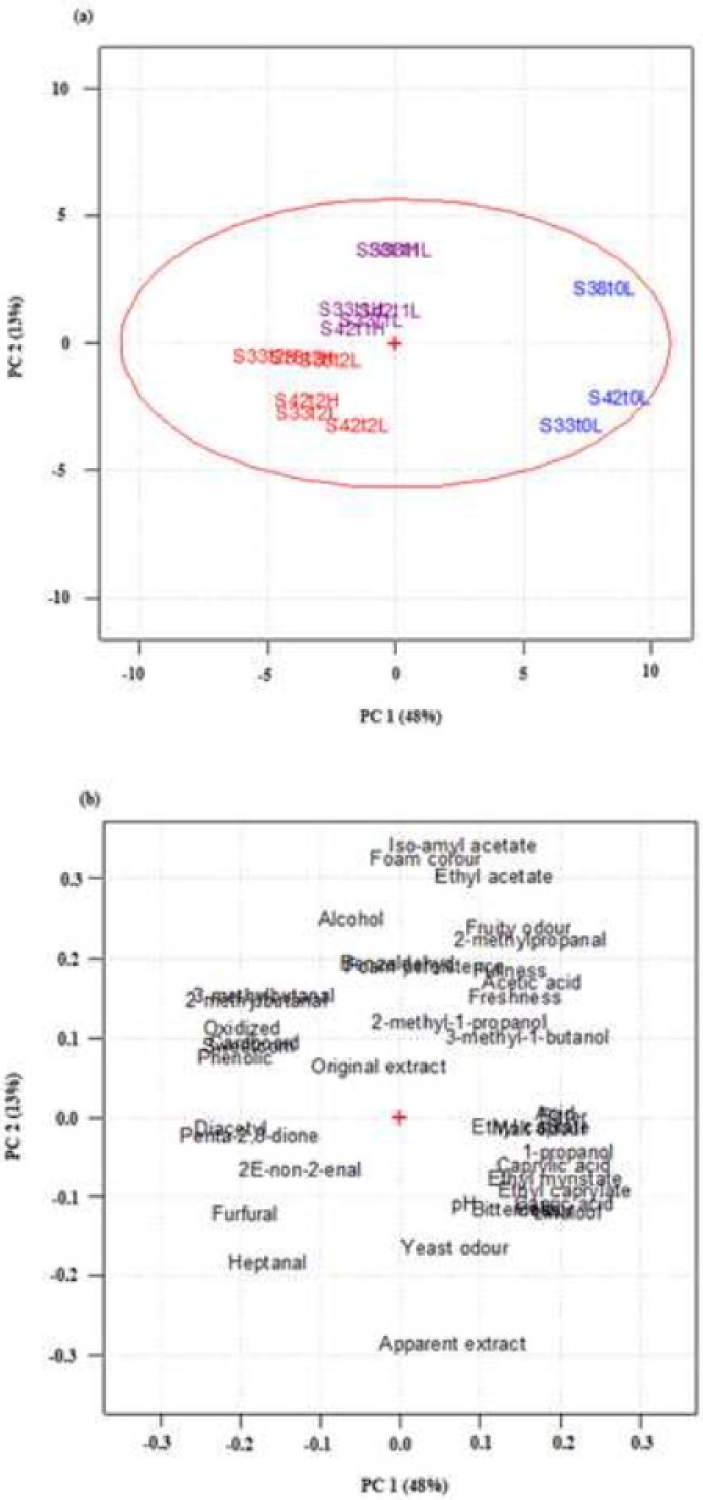

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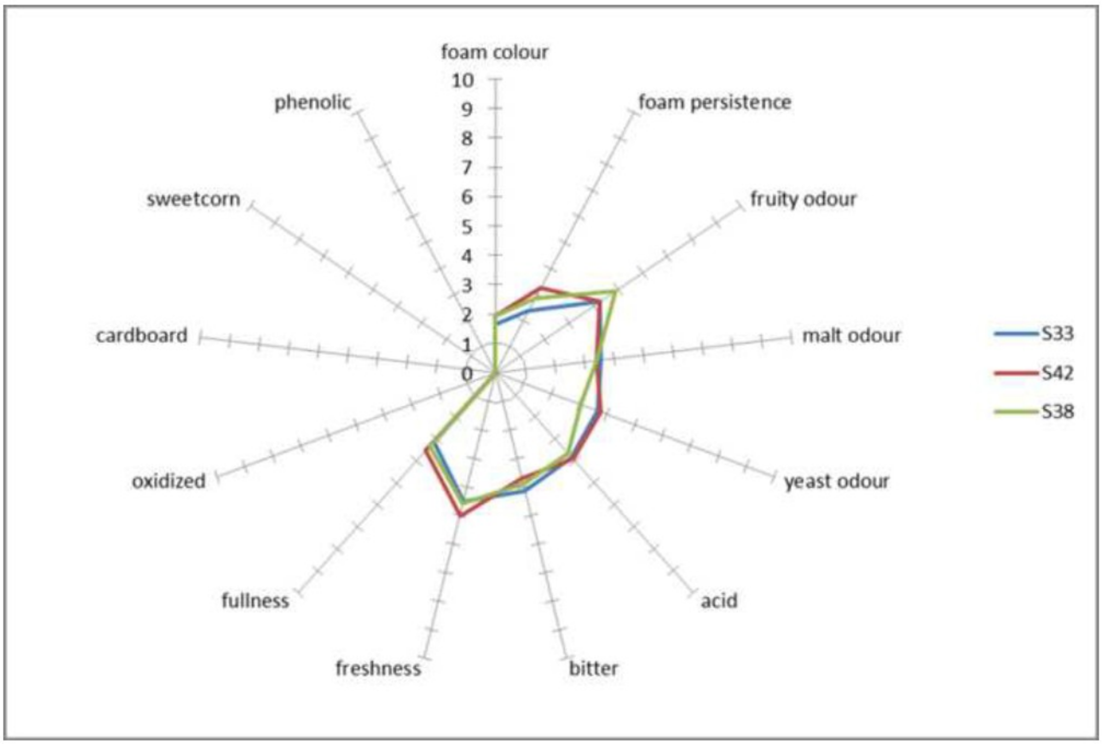

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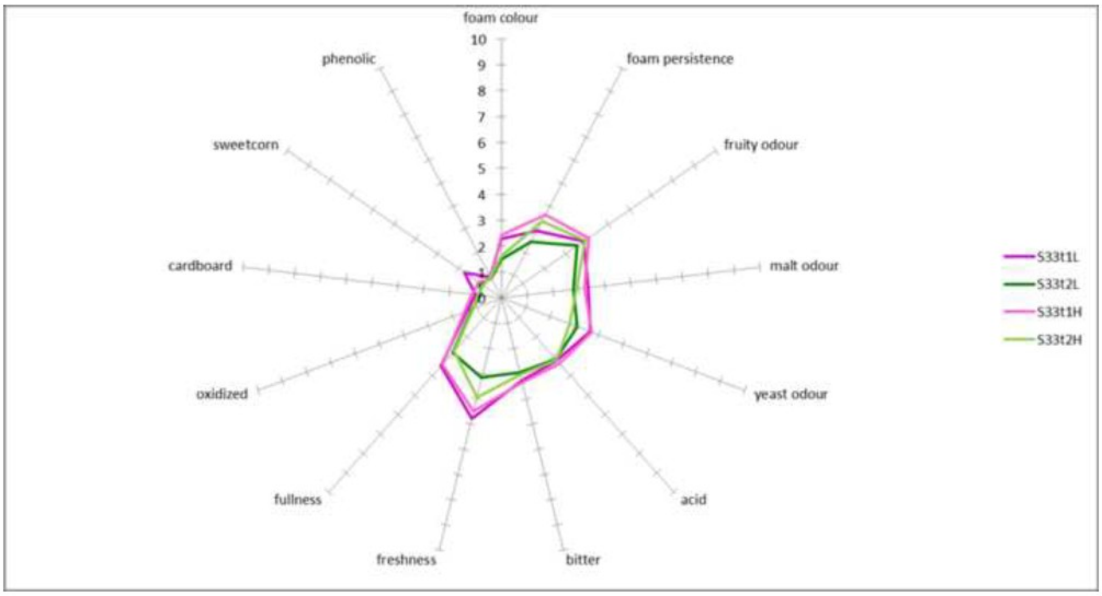

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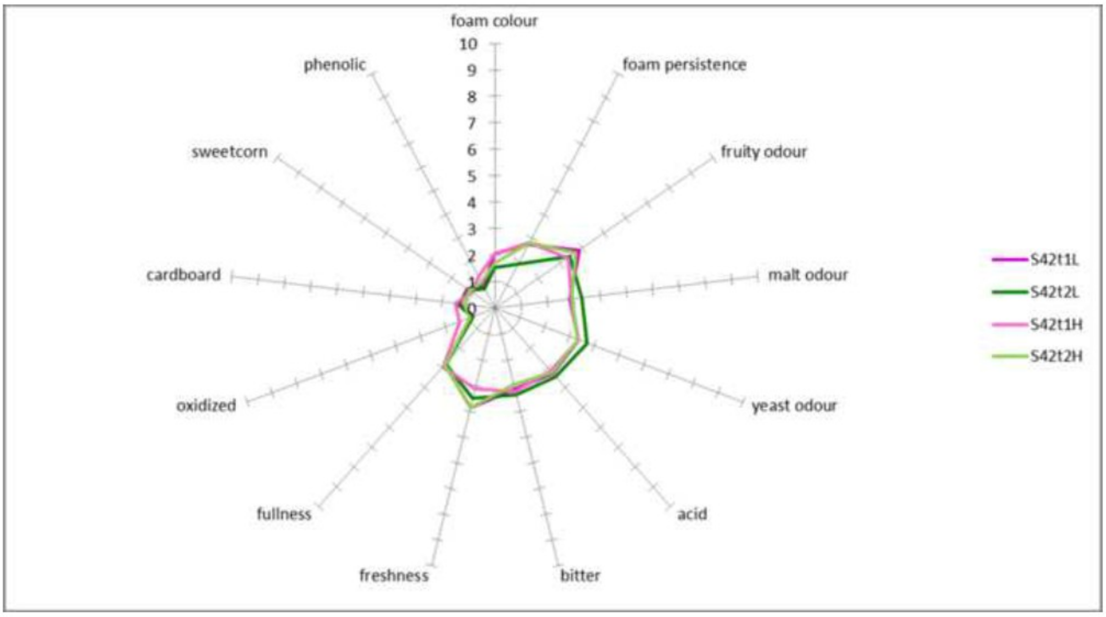

Fig. 5
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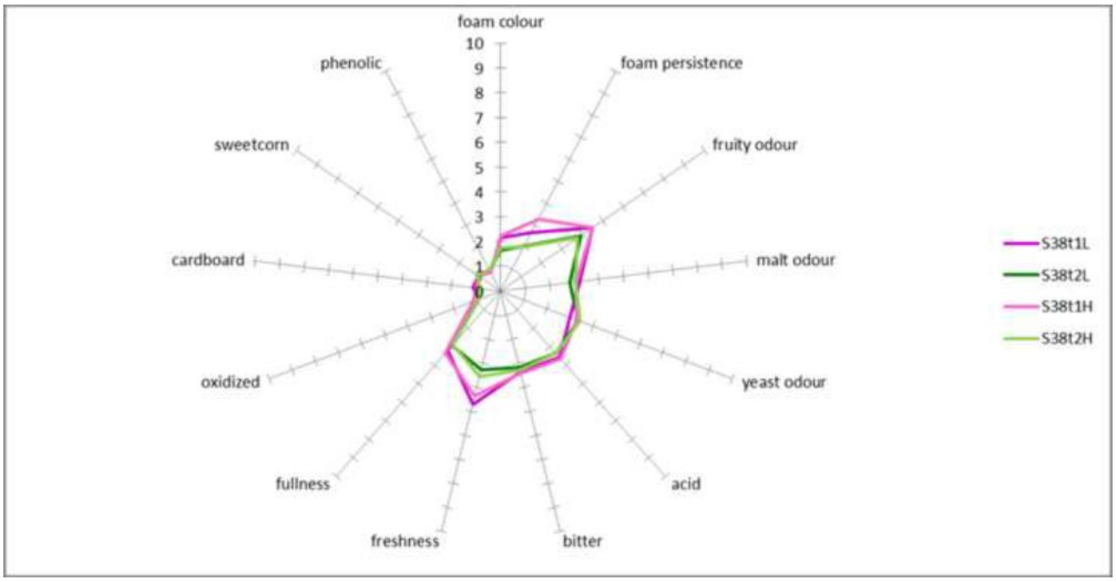

7 CONCLUSIONS

In the first work - “Preliminary characterization of an Italian craft durum wheat beer” - a Sardinian durum wheat beer was compared with two other standard wheat beers from different countries. The results of the real degree of fermentation were higher for the German beer, showing that fermentation occurred to a greater extent than for the other beers, and this was confirmed by the highest alcohol content and lowest pH of the German beer. However, the Sardinian beer had the highest values for original and real extracts, together with the highest polyphenol content. The Czech beer had the highest concentration of volatile esters and total alcohols. In sensory evaluations, the Sardinian beer was deemed to be more balanced in taste, and less sweet. This is the first comparative characterization of an Italian wheat beer, and in particular of a Sardinian craft durum wheat beer. More research will be required to develop correlations between sensory and volatile profiles with additional wheat beers, but this preliminary report offers some insights into Sardinian durum wheat beer.

In the second work - “Is it possible to create an innovative craft durum wheat beer with sourdough yeasts? A case study” - two Sardinian craft durum wheat beers, one fermented with a commercial yeast and the other with an autochthonous yeast, were compared. The values of real and apparent attenuation show that the fermentation has undergone to a greater extent in the beer produced with the autochthonous yeast. This is confirmed by the highest value of alcohol content and by the lowest value of pH in this beer. Probably due to this reason, this beer has an higher content of volatile substances respect to the beer produced with a commercial yeast. The results of the triangle test showed a significant overall difference between the two beers, while the paired preference test did not show significant difference for preference between them, even if it was observed a tendency to prefer the beer produced with the autochthonous yeast.

The use of autochthonous yeasts could provide an additional element of originality to the existing production, thus creating a further link with the territory that could have major repercussions from the economic point of view. In fact this is the first time that a beer with an autochthonous yeast isolated by sourdoughs has been produced. These interesting results could be the basis to enhance the originality of the Sardinian products with an “original” wheat beer and leading to the expansion of export markets outside the region.

Finally, in the third work - “Assessment of craft durum wheat beer during aging” - three Sardinian craft durum wheat beers produced in a microbrewery located in the south-east of Sardinia, were assessed during aging to evaluate the flavor stability on the basis of the variables yeasts, time, and temperature.
The volatile component, analyzed by GC-MS, changed over the period of six months with a decrease of esters and higher alcohols and an increase of carbonyl compounds. This trend is more evident at the highest temperature.
For what concerns the chemical-physical parameters, no significant differences were found regarding time, temperature or yeasts; only color resulted significantly different between the two temperatures, increasing at 28°C.
Finally, the sensory profiles showed a reduction of some attributes related to the flavor and in-mouth sensations during storage, confirming data obtained by GC-MS, but no significant differences were reported by the panel for the intensity of the off-flavors, even if the GC-MS analysis showed an increase of carbonyl compounds. No significant differences resulted regarding storage temperature. The difference in yeasts was found only for the fullness parameter, more intense in one of the autochthonous yeasts with respect to the commercial one.
In conclusion, the three beers, brewed with different yeasts, have similar chemical-physical, volatile and sensory characteristics during aging. This suggests the possible use of autochthonous yeasts in the brewing process, that could provide an additional element of authenticity to the existing production, in order to create a stronger link with the territory with positive economic rebound.
Here, it is presented, for the first time, the assessment of the behavior during aging of craft wheat beers. Future objectives are aimed at studying the shelf life of the beers for a longer period to improve the stability of the product with the main intent to investigate the possibility of expanding the market for craft beers outside of the Region, trying to enhance the originality of the Sardinia products.

8 OTHER WORKS

8.1. Oral presentation

The world of microbreweries in Sardinia and development of a craft durum wheat beer

Mascia, I.

INDC 2013, 13th International Nutrition & Diagnostics Conference, Olomouc (Czech Republic), 26-29 August 2013

L15 THE WORLD OF MICROBREWERIES IN SARDINIA AND DEVELOPMENT OF A CRAFT DURUM WHEAT BEER

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In recent years, local artisanal microbreweries have increased their production by marketing unique products that are very appreciated by consumers, whose demand for quality represents a challenge both for producers interested to improve their product in order to increase its appeal and for researchers involved in the characterization of new materials and in defining the best conditions for processing and storage the product.

Sardinia is following this trend so much that a dozen of microbreweries have arisen in the last years, but most of the malts on the market is imported. Every brewer uses malt, hop, water and yeast according to his taste, in order to obtain a unique product that has a powerful impact on an increasingly demanding market.

However, the use of local raw materials that could give the beer an additional value and connote it as strongly linked to the territory has to be yet investigated.

Currently our research is aimed at developing the local artisanal beer production through the selection and use of durum wheat and barley varieties grown in Sardinia. We will try to develop processing protocols suitable for obtaining higher quality wheat beers from the physical-chemical, sensory and nutritional point of view.

8.2. Poster

Is it possible to create an innovative beer with an old autochthonous yeast?

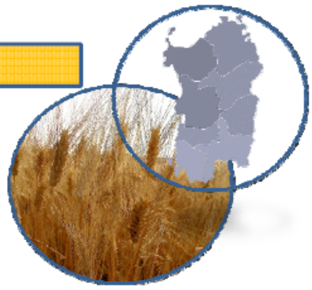
Mascia, I., Dostálek, P., Karabin, M., Fadda, C., and Del Caro, A.,

11th International trends in brewing, Ghent (Belgium), 13-17 April 2014

IS IT POSSIBLE TO CREATE AN INNOVATIVE BEER WITH AN OLD AUTOCHTHONOUS YEAST?

Ilaria Mascia[1], Pavel Dostálek[2], Marcel Karabin[2], Costantino Fadda[1], Alessandra Del Caro[1]
[1] Department of Agraria, University of Sassari, Italy
[2] Department of Biotechnology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Czech Republic

TARGET



Developing of the production of two local craft beers brewed with 20% of an old variety of unmalted durum wheat grown in Sardinia through the use of two different yeasts:

- a commercial one, normally used by brewers for wheat beers
- an autochthonous one, selected by the Microbiology section of the Department of Agraria (University of Sassari) from a Sardinian sourdough.

EXPERIMENTAL PART

Local craft beers for analysis: Two craft beers brewed in Sardinia, one with the commercial yeast and the other with the autochthonous yeast.

Chemical-physical analysis: apparent extract, real extract, alcohol content, original extract, apparent attenuation and real attenuation measured by the Anton Paar Alcolyser; total polyphenols, bitterness and pH, carried out according to EBC-methods (Analytica EBC, 1998) (Table 1). Results were analyzed with *t*-test ($p < 0.05$) using Statistica 6.0 for Windows, (values with different letters are significantly different at $p < 0.05$ by Student *t*-test).

Volatile analysis: extraction of volatile substances performed by headspace solid-phase microextraction coupled with GC analysis (HS-SPME-GC) (Table 2). Results were analyzed with *t*-test ($p < 0.05$) using Statistica 6.0 for Windows, (values with different letters are significantly different at $p < 0.05$ by Student *t*-test).

Sensory analysis: triangle test, performed with a panel of thirty judges, and paired preference test, performed with a panel of eighty judges. The triangle test was analyzed using the critical values table for triangle test, while the paired preference test was analyzed using the critical values table for paired difference test.

RESULTS

Table 1: *Chemical –physical analysis of wheat beers*

	Apparent extract (%)	Real extract (%)	Alcohol v/v (%)	Original extract (Plato)	Apparent attenuation (%)	Real attenuation (%)	pH	Bitterness (IBU)	Total Polyphenols (mg/l)
Autochthonous	3.70^{b}	5.49^{b}	4.99^{a}	12.99^{a}	71.5^{a}	57.7^{a}	4.30^{b}	23^{a}	169^{b}
Commercial	4.10^{a}	5.81^{a}	4.75^{b}	12.93^{a}	68.4^{b}	55.1^{b}	4.81^{a}	23^{a}	185^{a}

Table 2: *Volatile analysis of wheat beers*

	Ethyl acetate (mg/l)	1-Propanol (mg/l)	Isobutanol (mg/l)	Isoamyl acetate (mg/l)	Isopentanol (mg/l)	Acetic acid (mg/l)	2-Phenylethanol (mg/l)	Caprylic acid (mg/l)
Autochthonous	15.88^{a}	5.25^{b}	17.33^{a}	1.24^{a}	21.71^{a}	6.83^{a}	15.10^{a}	4.35^{a}
Commercial	12.40^{b}	9.60^{a}	7.39^{b}	0.86^{b}	11.63^{b}	6.41^{a}	15.47^{a}	3.52^{b}

CONCLUSIONS

The values of real and apparent attenuation show that the fermentation has undergone to a greater extent in the beer produced with the autochthonous yeast. This is confirmed by the highest value of alcohol content and by the lowest value of pH in the beer produced with the autochthonous yeast. In fact, in the fermentation process, ethanol and acids (such as citric and acetic acids) are produced, increasing alcohol content and lowering pH. Probably due to this reason, the beer produced with the autochthonous yeast has an higher content of volatile substances respect to the other beer. For what concern the polyphenolic content, it is higher in the beer produced with a commercial yeast. The bitterness content is not significantly different between the two beers. The results of the triangle test showed a significant overall difference between the two beers, while the paired preference test did not show significant difference between them, even if it was observed a tendency to prefer the beer produced with the autochthonous yeast.

WHAT'S INNOVATIVE

The possible use of autochthonous yeast could provide an additional element of originality to the existing production, thus creating a further link with the territory that could have major repercussions from the economic point of view. This is important to create the basis for the expansion of export markets outside the region, trying to enhance the originality of the Sardinian products.

Acknowledgement

Ilaria Mascia gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 - Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.) and Ministry of Agricultural, Food and Forestry Policies (MIPAAF) for the financial support of "PROBIRRA" OIGA III project.

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9 ACKNOWLEDGEMENTS

First, I would like to thank my tutor Dr Alessandra Del Caro who has always believed in my ideas and has inspired me to achieve all my goals, including the European doctorate. Thanks for your teachings that make me grow as a researcher. Thanks for your great availability, for your helping all the times, including Saturdays and Sundays!!!

I would like to thank Professor Pavel Dostalek for having given me the possibility to work in the Institute of Chemical Technology in Prague, for having introduced me in the world of beer with his vast experience, and for having made available all the instrumentation that made the realization of my thesis possible.

I would like to thank Dr Costantino Fadda for the help, advices, and for having supported (and "sopportato") me in these three years. Thanks for having borne all my mood changes and for having always found the solutions to my doubts!!!

I would like to thank the Lara micro-brewery for the technical help provided during the project, without which I could not get my results.

I would like to thank Professor Marilena Budroni for her availability and her affection.

I would like to thank Veronica Bellucco and Emilio Mouannes, with whom I shared many moments of this PhD thesis.

I would like to thank Professor Antonello Piga, Dr Vincenzo Vacca, Paolo Fenu, Giampaolo Usai, Paola Conte, Anna Maria Sanguinetti and Paolo Urgeghe for having supported me during my research work and for having created a beautiful working environment which is usually hard to find.

I would like to thank Giangiacomo Milella, officemate and friend, for his availability and for the help he gave me in every moment.

I would like to thank all the sensory panel: Giampaolo Usai, Marc Bou Zeidan, Giovanni Demuru, Sara Landolfo, Yolanda Matia, Antonio Mazza, Chiara Multineddu, Cinzia Porru, Salvatorico Razzu, Antonella Saba, Silvia Tanda, and Alessio Vargiu for the kind collaboration.

I would like to thank my parents and my brother for being a no-stress factor with their encouragement and their patience.

To Marco. The only fact that you exist makes everything easier. You know.