Development of Strategies for Whisky Production Applying Shorter Ageing Periods

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e do **Doutor Eduardo João Louro Coelho**

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STATEMENT OF INTEGRITY

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Abstract

Development of strategies for whisky production applying shorter ageing periods

Whisky is one of the most appreciated drinks in the world. The legislation that defines the minimum age in contact with wood of this distillate restricts the application of methodologies that have been developed in order to make the process more efficient, faster and economical. The idea that a prestigious whisky must be produced in a traditional way is still very much ingrained. However, some scientific studies have shown that the application of new technologies, techniques and optimization of processes are extremely effective in controlling the production of this alcoholic beverage allowing for a purer distillate and maintaining the desired organoleptic profile, similar to the traditional one.

This thesis has the objective of developing a grain spirit similar to a single malt whisky optimizing the process related to the production of this distillate including: testing of different malts as well as testing the performance of two different yeast strains; evaluating the effect of clarification and lagering processes; carrying out the fractional distillation of the fermented products applying a different approach to mixing the different fractions and developing accelerated maturation methodologies by using American oak chips previously put in contact with fortified wine at high temperature with agitation.

We were able to select the most adequate malt and yeast to obtain both the desired organoleptic profile and the alcohol concentration for the fermented product and the final distillate. The shorten maturation also resulted positively as the grain spirits presented compounds typical of wood and wine and when evaluated by a sensory panel received positive scores in visual, olfactory and taste profiles being consensual that it's a quality product and with potential to be commercialized.

Also, as exploratory trials, distillations of Letra D and Letra E were performed with some additions such as hops in the distillation system. The spirits produced were evaluated by the same panel and the results supported further experiments integrated in the internship at FermentUM which culminated in the release of two commercial products as part of Letra Craft Trials.

Keywords: accelerated maturation; distillation; grain spirit; sensory profile; whisky

Resumo

Desenvolvimento de estratégias para a produção de whisky com redução do tempo de maturação

O whisky é uma das bebidas mais apreciadas no mundo. A legislação que define o período mínimo de contacto com madeira para este destilado restringe a aplicação de metodologias que foram desenvolvidas com o intuito de tornar este processo mais eficiente, rápido e económico. A ideia de que um whisky de prestígio deve ser produzido de forma tradicional ainda se encontra muito enraizada. No entanto, estudos científicos demonstram que a aplicação destas tecnologias, técnicas e otimizações de processos são extremamente eficazes a controlar a produção desta bebida alcoólica permitindo um destilado mais puro e mantendo o perfil organolético desejado, semelhante ao tradicional.

O objetivo desta tese é a produção duma bebida espirituosa de cereais semelhante a um whisky *single malt* otimizando os processos relacionados à produção do destilado, incluindo: testar tanto diferentes maltes como a performance de duas diferentes estirpes de levedura; avaliação do processo de clarificação e de *lagering*; levar a cabo a destilação fracionada dos produtos fermentados, aplicar uma diferente abordagem à mistura das diferentes frações e o desenvolvimento de metodologias para maturação acelerada usando *chips* de carvalho Americano previamente colocadas em contacto com vinho fortificado a alta temperatura e com agitação.

Conseguimos selecionar o malte e a levedura mais adequados para conseguir obter o perfil organolético desejado, para as concentrações de álcool pretendidas para o produto fermentado e para o destilado final. A maturação acelerada resultou positivamente pois as resultantes bebidas espirituosas de cereais apresentaram compostos característicos da madeira e do vinho e quando apresentadas e avaliadas por painel sensorial que classificou positivamente as amostras tanto a nível visual, olfatório e de paladar sendo consensual tratarem-se de produtos de qualidade e com potencial comercial.

Adicionalmente, enquanto ensaios exploratórios, foram feitas destilações de Letra D e Letra E com múltiplas variações tais como: contacto com lúpulo no aparelho de destilação. As bebidas espirituosas foram avaliadas pelo mesmo painel e os resultados suportaram experiências subsequentes integradas num estágio na FermentUM que culminou no lançamento de dois produtos comerciais como parte da gama *Letra Craft Trials*.

Palavras-chave: Whisky; bebida espirituosa de cereais; destilação; maturação acelerada; perfil sensorial

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List of abbreviations, variables and acronyms

ABV – Alcohol by Volume

AD – Anno Domini (in the year of the Lord)

EU – European Union

SM – Single Malt

SG – Single Grain

EC – European Community

EEC – European Economic CommunityEt al. – et alii/et aliae/et alia (and others)

FAN – Free Amino Nitrogen

SMM – S-methyl methionine

DMSP – Dimethylsulfidesulfoniopropionate

DMS – dimethyl sulphide

pH – potential of hydrogen

e.g. – exempli gratia (for example)

Pi – Pilsner

Pa – Pale Ale

Vi – Vienna

sp – species

YPD – Yeast Extract Peptone Dextrose

Cf – Final concentration

Ci – Initial concentration

Vi – Initial Volume

Vf – Final Volume

HPLC – High performance liquid chromatography

GC-FID – Gas Chromatography with Flame-Ionization Detection

GC-MS – Gas chromatography–mass spectrometry

GM – Geometric Mean

C_{c02} – Concentration of Carbon Dioxide

t – time

BA – Belgian Abbey

s33 – Fermentis SafAle S-33

Lda – Limitada

PT – Perception Threshold

Objectives

The main objective of this thesis is the production of a grain spirit, similar to a Single Malt Whisky, from barley malt applying short ageing periods. This thesis aims to study and optimize all steps of the production process of this type of distillate in order to accomplish all required technical parameters and obtain a beverage with the desired organoleptic profile.

The specific objectives oh this study:

the production of a "single malt" wort with the desired fermentable sugar content;

fermentation of the wort for the production of a fermented product with 10% of alcohol by volume

definition of a distillation methodology for the production of a spirit with an ethanol content (> 50% (v/v)) while minimizing the characteristic chemical defects of distillates

application of accelerated maturation for the inducement of the desired sensorial character for the distillate (application of American oak chips previously in contact with fortified wine and the definition of the different contact variables more favorable to the transfer of the extractive fraction from the wood to the drink)

the production of a final product with alcohol content of a traditional single malt (40% (v/v)) and organoleptic profile similar to a barrel aged whisky;

By reaching these aims this thesis intends to shed light over processes that are innovative and have high industrial application for the distilling industry to reduce costs and time, increase production output and help to create new high-quality products based in non-traditional methods that are proven to have commercial potential.

1. Introduction

1.1. Whisky's history

Whisky, or Whiskey, is an alcoholic beverage derived from the distillation of fermented grain mash, usually aged in wood casks and diluted. Distilled malt whiskies on average contain 70% alcohol by volume (ABV) although, after maturation, some loss due to evaporation takes place reducing the alcohol content to between 55% and 65%, which is further decreased by dilution to around 40%. These products are usually classified in accordance to their ingredients, origin, ageing and blending (Karlsson & Friedman, 2017; Power et al., 2020).

Although distilling dates back to the 1st century AD, the earliest records of alcoholic distilling were traced back to the distillation of wine in 12th century Italy. Henceforth, the technique spread across Europe, where the belief that distilled drinks had medicinal properties led to their moniker "aqua vitae" (the water of life). In 1494 Friar John Corr, a craft distiller, was granted the first license to produce these beverages, as they reached the British Isles, paving way to the distillation of the first whiskies leading to "uisge beatha", the local name for this drink, which gave root to the current day word, Whiskey (Ireland and America) or Whisky (Scotland) through phonological change from "uisge", "usky", and eventually to "whisky" (M. Jackson, 2004; Pawlaczyk et al., 2019)

Although the designations "Scotch Whisky" and "Irish Whiskey" expand beyond mere geographical indicators, these nomenclatures are internationally recognized and protected under Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012. The status of these products coupled with price and relative scarcity create a strong incentive for counterfeit products, to be provided to unsuspecting consumers, which not only risks market perception, due to their low quality, but also endangers public health and safety. As such, continuous monitoring and development of new methodologies to protect consumers are required, leading to high investment in consumer safety and protection (Piggott, 2015; Power et al., 2020).

The three more prominent whiskies are: Scotch whisky, Irish whiskey and American whiskey. Scotch whisky is subjected to maturation periods of at least three years (introduced during WWI to try to curb inebriation amongst ammunition factory workers) in oak casks and must be produced by a Scottish distillery from one of the following regions: Campbeltown, Highlands, Islay, Lowlands and Spayside. These beverages are categorized according to their production process under one of the following categories: Single Malt - SM (malted barley only), Single Grain – SG (malted barley with or without whole grains of other malted or unmalted cereals such as wheat, maize or barley), Blended (a blend of one or more SMs

with one or more SGs), Blended Malt (a blend of SMs distilled at more than one distillery) and Blended Grain (a blend of SGs distilled at more than one distillery). Irish whiskey is also aged for at least three years and is a product derived from a grain bill that consists of at least 25% malted barley, being that it may contain a mixture of other grains that can either be unmalted or malted, with the drying process taking part in closed kilns instead of taking place over open peat fires. Furthermore, these spirits are subjected to a triple distillation. American Whiskey must be matured in a charred new oak vessel, being that the drink itself is defined as a distillate of a fermented grain mash with less than 95% alcohol. It can be categorized as bourbon, corn, pure malt, rye, rye malt or wheat. Overall these spirits can be divided into malt whisky, grain whisky and blended whisky (M. Jackson, 2004; Pawlaczyk et al., 2019; Piggott, 2015; Power et al., 2020).

1.2. Legal Framework

For the purpose of this thesis, it is important to strictly differentiate Whisky from grain spirits. That distinction is established under article 2 and article 3, Annex II, (a) of (Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the Definition, Description, Presentation, Labelling and the Protection of Geographical Indications of Spirit Drinks and Repealing Council Regulation (EEC) No, 2008) which repealed European Community Council (ECC) regulation no. 1576/89 and which states:

2. Whisky or Whiskey

(a) Whisky or whiskey is a spirit drink produced exclusively by:

(i) distillation of a mash made from malted cereals with or without whole grains of other cereals, which has been:

— saccharified by the diastase of the malt contained therein, with or without other natural enzymes,

— fermented by the action of yeast;

(ii) one or more distillations at less than 94.8 % vol., so that the distillate has an aroma and taste derived from the raw materials used,

(iii) maturation of the final distillate for at least three years in wooden casks not exceeding 700 liters capacity.

The final distillate, to which only water and plain caramel (for coloring) may be added, retains its color, aroma and taste derived from the production process referred to in points (i), (ii) and (iii).

(b) The minimum alcoholic strength by volume of whisky or whiskey shall be 40 %.

(c) No addition of alcohol as defined in Annex I(5), diluted or not, shall take place.

(d) Whisky or whiskey shall not be sweetened or flavored, nor contain any additives other than plain caramel used for coloring.

3. Grain spirit

(a) Grain spirit is a spirit drink produced exclusively by the distillation of a fermented mash of whole grain cereals and having organoleptic characteristics derived from the raw materials used.

(b) With the exception of 'Korn', the minimum alcoholic strength by volume of grain spirit shall be 35 %.

(c) No addition of alcohol as defined in Annex I (5), diluted or not, shall take place.

(d) Grain spirit shall not be flavored.

(e) Grain spirit may only contain added caramel as a means to adapt color.

(f) For a grain spirit to bear the sales denomination 'grain brandy', it must have been obtained by distillation at less than 95 % vol. from a fermented mash of whole grain cereals, presenting organoleptic features deriving from the raw materials used.

The final product obtained as a result of this study is legally framed as a grain spirit.

1.3. Malt whiskies - Raw materials

1.3.1. Barley

The major producers of malt whisky are Scotland, Ireland, India and Japan of whom all use malted barley as their main ingredient. The botanical name for barley, whose grain structure is present in Figure 1, is *Hordeum vulgare* which is a member of the grass family Poaceae. Evidence suggests that barley was domesticated in the Fertile Crescent in the Middle East, as early as the Neolithic times. Cultivation in Iran dates to 8000 BC and in ancient Egypt gained importance due to its popularity for both brewing and baking (Badr et al., 2000; Baik & Ullrich, 2008; Dolan, 2003).

In present day it amounts to 12% of the global crop cultivation, being the fourth most popular crop behind only wheat, rice and maize. As it's a crop that is very resistant to both winter and drought conditions, as well as maturing early, it reveals itself as being more economical to cultivate. Around 33% of all the cultivated barley is destined to malting (Idehen et al., 2017). Barley's genetic variety mirrors its diversity

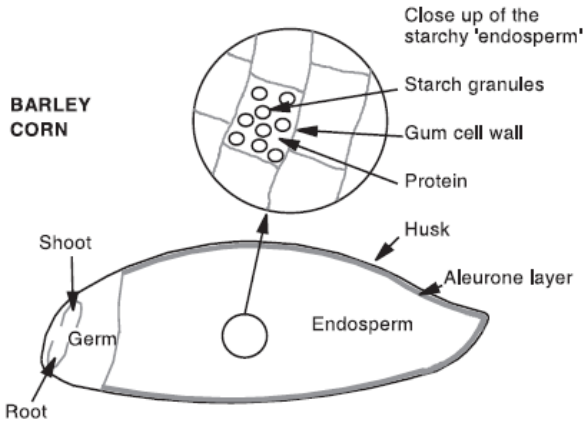

Figure 1: Barley grain structure (Dolan, 2003)

and, as such, it can be classified according to the disposition of the kernels along the spike (2-row or 6-row), seasonality (spring or winter), presence or absence of a hull strongly clinging to the grain (hulled or hulless) and end-use (malting or feed). A barley kernel has an average moisture content of 13% to 15%, the remaining content is referred to as the "dry matter" which is mainly composed of carbohydrates, proteins, inorganic matter, lipids and other substances such as polyphenols. Over 70% of the dry matter of barley is made up of carbohydrates which can be categorized as storage carbohydrates (mostly starch) and structural carbohydrates (cellulose and hemicellulose) (Baik & Ullrich, 2008; Boulton, 2013).

The glucose formed via photosynthesis is transported upwards via the stem to the endosperm of maturing grains and gets polymerized into starch, which is stored in granules (small or large) also known as amyloplasts, that contain two different types of starch: amylopectin (which represents 80% of the starch content, is comprised by glucose molecules connected via α1-4 and α1-6 glycosidic bonds which are responsible for the branched structure which in turn leads to a lower gelatinization temperature) and amylose (long, unbranched helical chain of α1-4 linked glucose molecules). Regarding the structural carbohydrates, present in the cell walls as microfibers: cellulose (a linear polymer of D-glucose units connected via β1-4 glycosidic bonds, primarily located in barley husks that for being a water-insoluble molecule plays a major role during wort filtration) and hemicellulose, the main compound (75%) of the endosperm cell walls, which can also be found in the husks, consists of β-glucans and pentosans. When presented as microfibers, β-glucans are unbranched linear chains of D-glucose monomers connected via β-(1-3) and β-(1-4) bonds, which are broken down by β-glucanases during germination by a process called cytolysis, important to make starch available for hydrolyzation by amylase. Barley pentosans are mostly arabinoxylan polymers consisting of β1.4-D-xylose linked backbone, to which arabinose residues are occasionally bound via α1.2 or α1.3 bonds. During the malting process arabinoxylan needs to be partially

broken down as to allow the diffusion of hydrolytic enzymes from the aleurone layer towards the endosperm (Boulton, 2013; Djurle et al., 2016; Lynch et al., 2016; Priest & Stewart, 2006; Vriesekoop et al., 2010).

1.3.2. Water

Although much attention is given to the grain, water mustn't be neglected as one of the main ingredients in spirits production as it plays key roles in three stages of the process: malting, mashing and dilution (aside from also often being used in the heat distribution – both cooling and heating – and cleaning of the plant). In fact, the whole process from earth to bottle involves adding and subtracting water, be it in irrigation of the crops upon sowing, removal of water through drying for storage of the grain, addition in steeping and removal in kilning and further onto other processes that will be expanded later (Dolan, 2003).

1.3.3. Yeasts

Yeasts are fungi that predominantly grow as unicellular beings, although some species exhibit dimorphism and present stages in their life cycle in which they adopt filamentous morphology and develop mycelium. Brewing and distilling strains belong to the genus *Sacharomyces* (Latin word for "sugar fungus") being the most important species *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* are anaerobic facultative heterotrophic organisms and thought of as domesticated yeast strains which include those used for brewing, baking enology and production of spirits. The genome of *S. cerevisiae* has been under close attention for decades being one of the most thoroughly understood of all organisms, as this knowledge has important applications in fermentation technology. For the adequate growth of yeast suitable pH, temperature and nutrients are required. As fungi are classified as ascomycetes for producing ascospores through meiosis (sexual reproduction). The sexual reproduction takes place when yeasts are subject to nutritional stress, still, they also can reproduce asexually by budding or fission. Vegetative reproduction is normal under the conditions found in grape must and wine as ascal development is suppressed by high concentrations of glucose, ethanol or carbon dioxide (Albergaria & Arneborg, 2016; Davydenko et al., 2020; Gallone et al., 2018; Knop, 2011).

1.4. Malt Whisky – Production process

1.4.1. Malting

The malting process consists of several stages in which the unmalted grains undergo germination under controlled conditions in order to produce the malt that contains starch, proteins and other components

together with a package of enzymes required for their degradation. These stages comprise cereal cleaning, sorting and storage, steeping, germination, drying and kilning and deculming. The raw materials for malting are usually cereal grains of which barley is the most common, although other grains such as oat, rye and rice are also used. These products have various uses in the food industry but in the context of brewing and distilling, malts are used as source of fermentable sugars and a wide range of other nutrients which will be used by the yeast to grow during the fermentation stage (Boulton, 2013; Müller & Methner, 2015).

1.4.1.1. Steeping

The goal of steeping is to promote the uniform germination and hydration of the endosperm to a point where it is suitable for modification (sum of the processes that occur within the starchy endosperm tissue of cereal grains). For barley, steeping is recommended to last between 40 and 60 hours at a temperature of 14 °C reaching a final humidity of 45% (Ispiryan et al., 2021).

1.4.1.2. Germination

The purpose of germination is to maximize fermentable extract by promoting both endosperm modification and the development of hydrolytic enzymes. The products of enzyme activity are mainly sugars (from partially degraded starch), amino acids, smaller concentrations of various salts and metal ions which accumulate within the endosperm providing nutrients for the development of the embryo. The hydrated grains are allowed to germinate under strictly controlled conditions of temperature and humidity. During this stage barley kernels develop rootlets and an acrospire being, at this point, called chitting barley. To avoid rootlets matting together mechanical agitation is applied. Adequate ventilation is also required to disperse the heat produced by the aerobic respiration (Frank et al., 2011; Lynch et al., 2016; Müller & Methner, 2015).

To make starch accessible for enzymatic degradation its necessary that partial degradation of the endosperm cell walls (cytolysis) occurs. For this to happen, enzymes involved in the breakdown of β-glucans and pentosans need to be active. Proteolytic enzymes such as peptidases break down high molecular weight proteins into soluble low molecular weight molecules like oligopeptides and amino acids. It is important to ensure that Free Amino Nitrogen (FAN), the measure of low molecular weight substances, mainly amino acids, has proper levels as to not limit yeast growth and metabolism during fermentation (Fang et al., 2019; Lekkas et al., 2005; Müller & Methner, 2015).

1.4.1.3. Drying and Kilning

The goal of drying and kilning is to halt biological activity of freshly produced malt (green malt) after the required degree of modification and enzyme levels have been reached, in order to produce a dry storable product. In order to reach this goal, the water content of green malt is reduced from 42 – 48% to between 3 – 6%, stopping enzymatic activities while forming color and flavor compounds (Müller & Methner, 2015). Drying and kilning are divided into a subset of different stages, during which the temperature of the incoming air is controlled. Drying takes place until a decrease of the relative humidity and a rise in the temperature of the outgoing air is observed and "breakthrough" is said to be reached. At this point the intake air temperature is further increased leading to the kilning phase where the temperature reaches 80 °C to 105 °C, depending on the desired type of malt, and until the water content of the malt reaches less than 6%. When the kilning time is longer and at higher temperatures, a malt darkening is achieved resulting, in some cases, in a marked reduction of enzyme activity. These malts are used because of their coloring, flavor and aromatic properties (Boulton, 2013; Priest & Stewart, 2006).

After the breakthrough, aside from the aforementioned decreased in enzymatic activity, also occurs the conversion of S-methyl methionine (SMM), a precursor for dimethylsulfidesulfoniopropionate (DMSP), into dimethyl sulphide (DMS), a volatile compound responsible for a "cooked vegetable" off-flavor. Part of DMS is oxidized to dimethyl sulphoxide that can, later on, be reduced back to DMS by yeasts. Maillard reactions also take place both during kilning, wort boiling and distilling. At temperatures higher than 90 °C, primary amines (mainly amino acids) and carbonyls (mainly reducing sugars, aldehydes and ketones) react to form organic color and flavor compounds. These reactions can be subdivided into three steps: reaction of the reducing sugar with amino acid producing an unstable N-substituted glycosamine; formation of ketosamine due to the isomerization and by Amadori rearrangement of the gycosamine; the ketosamine can react differently depending on the conditions to produce a wide variety of products such as, fission products (ethanol, diacetyl, pyruvaldehyde), furfural, dehydrofurfural and hydroxymethyl furfural (giving malty and caramel flavors), reductones and dehydroreductones (that can react with amino acids forming Strecker aldehydes, usually regarded as off-flavors); melanoidins (pyarazines and pyrroles, responsible for the dark colors of roasted malts). After kilning, the malt is cooled and the rootlets are removed (deculmed) being that the now separate rootlets can be used for animal feed while the cleaned malt is stored (Lee et al., 2001; Priest & Stewart, 2006).

During barley kilning, peat (decayed plant material generally found in wetland areas) can be burnt avoiding flaming to produce a smoke called "peat reek" which is an extraneous source of distinct flavor to whisky due to phenolic compounds (Bathgate et al., 1977; Hayes et al., 2020).

1.4.2. Milling

The main objective of malt and cereal milling is to turn the kernels into finely ground and milled particles. By reducing their size to smaller particles, the best yield of extract can most easily be obtained in the next step, the mashing, as it facilitates water penetration of the cereal endosperm, allowing enzymes to have a larger surface area to act. By milling, starch granules are mechanically damaged which promotes the absorption of water and facilitates the release of starch from the protein matrix of the malt, reducing the gelatinization temperature while at the same time helping to break down gums such as arabinoxylans and β-glucans and promoting solubilization of proteins. The product that derives from the milling is called grist. Depending on the type of wort filtration applied, different milling techniques are used. Generally, if a traditional lauter tun is used to filter the wort, the main objective is to ground the inner kernels as much as possible, often with roller mills, while avoiding damage to the husks, as intact husks are crucial to form the filter bed. If instead filtration is done resorting to a mash filter, where wort is filtered using a very fine pore polypropylene cloth instead of a husk-dependent filter bed, a more destructive way of milling, for example a hammer mill, can be applied that will increase the fraction of smaller particles in the grist resulting in a higher yield of malt extract (Boulton, 2013; Djurle et al., 2016; Piggott, 2015).

1.4.3. Mashing

Grist and hot water are mixed (in a ratio of 2.15-2.42hL per 100Kg of grist) in a process called mashing, resulting in the solubilization of the malt constituents and the action of malt-derived enzymes on the resulting mash. The most important enzymatic reactions that take place are β-glucan degradation, protein breakdown, starch degradation and conversion of fatty acids. The mash is kept at specific temperatures and pH levels (between 5.2 and 5.5) for about an hour to ensure proper enzymatic conversion. Individual enzymes may be sequentially inactivated either due to heat denaturation, via the increase in concentration of inhibitors via selective proteolysis. As already mentioned in the malting process, the breakdown of cell walls is one important phenomenon to be considered during mashing. As such, in high quality malt most of the high molecular weight β-glucans have already been degraded into low molecular weight β-glucans, and 90% of β-glucanases are inactivated during kilning. Still, these processes must be taken into account during mashing as, with poorly modified malt, the viscosity of the mash is increased at temperatures above 50 °C due to the β-glucans solubilase continuing to convert insoluble high molecular weight β-glucans into soluble high molecular weight β-glucans which can cause problems in the filtration (Piggott, 2015; Priest & Stewart, 2006).

Regarding to the protein breakdown there are two major types of proteolytic enzymes in malt: endopeptidases, that generate high molecular weight peptides and exopeptidases that act on the outer ends of a peptide generating single amino acids (yeast nutrients). Unlike traditional mashing techniques in which the starting temperature is between 45 °C and 55 °C (optimal temperature range in which proteases show highest activity and degrade proteins to short peptides and amino acids, forming the major nitrogen source for yeast during fermentation), modern highly-modified malts can be mashed directly at temperatures over 60 °C as the protein breakdown has already been completed by the maltster (Boulton, 2013; Lekkas et al., 2005).

Starch degradation can be divided into three stages: gelatinization (process of swelling and hydration of starch granules with hot water so that the starch loses its crystalline state becoming amorphous and soluble in water allowing enzymes to directly act on it, barley malt starch gelatinization is initiated at 59 – 65 °C); liquefaction phase (the starch amylose and amylopectin molecules are hydrolysed into smaller chains, mostly by α-amylases) and saccharification (α-amylase progressively degrades amylose and amylopectin molecules into short dextrines, β-amylase will act on the non-reducing ends that are created, splitting off maltose, glucose and maltotriose and limit dextrinase which is able to hydrolyse the 1.6-bond present in amylopectin) (Balcerek, 2016; Briggs et al., 2004).

The iodine test (for example, Lugol's iodine solution) is used to evaluate the progress of starch breakdown and subsequent release of fermentable sugars during mashing. Iodine solutions react with helices formed by the long chains of glucose monomers that are present in amylose and amylopectin molecules in starches. The test is performed by taking a sample of the mash and adding a few drops of iodine solution and observing the color change and its intensity. The complex of iodine together amylose results in a deep blue-black color while complex with amylopectin leads to a red-violet color. Conversion is completed when the natural yellowish color of the iodine doesn't change when it's mixed with the mash sample, which is considered a negative result. Lipid inclusions naturally present in starch prevent the reaction with iodine can lead to an underestimate of the saccharification progression which may be surpassed by adding a solvent such as butanol (Briggs et al., 2004).

The fat degrading process started during malting, in which malt-derived lipids are degraded into fatty acids by lipases, is continued during mashing by lypoxygenases which convert mainly unsaturated fatty acids into hydroxy fatty acids and glycerine being the former, in the presence of oxygen, precursors to compounds known as aging carbonyls that can introduce some undesired off-flavors such as "cardboard" and "wet paper" aroma descriptors which is why it is important to reduce or avoid the oxygen uptake at this stage of the production (Briggs et al., 2004).

1.4.4. Lautering

The filtration process where the soluble part of the mash (liquid also called wort) is separated from the insoluble part (husks and other materials) is called lautering. After mash out, the liquid is removed from the bottom of the mash and added to the top, this way recirculating the wort so that insoluble particles present below the grain bed can be filtered out of the liquid wort. After the recirculation is complete and the liquid is free from solids, the wort can be channeled to the boiling vessel leaving only the solids with residual sugars and other nutrients behind. Afterwards, more hot water (heated to, between 75 – 78 °C) is added to the grain bed in a step called sparging where the residual nutrients are washed and added to the remaining wort in the boiling vessel. The most widely used systems for this process are the lauter tun and the mash filter (Priest & Stewart, 2006).

1.4.5. Wort Boiling

After lautering the wort is boiled, being steam heating the most commonly used way to increase wort temperature. There are several goals expected from this stage, crucial to obtain a quality product: boiling sanitizes the wort as it eliminates most microorganisms, although the process doesn't kill spores that are extremely heat resistant; polyphenols present in wort can bind to high molecular weight proteins and precipitate, being these complexes called hot trub since they result in visible break flakes (increased boiling time, low pH and agitation of the work stimulates this precipitation); coagulation of protein material (once more it's important to measure FAN levels to ensure that further ahead in fermentation yeasts have sufficient nitrogen sources); inactivation of the enzymes occurs because of the high temperatures during this stage that cause proteins to denature losing their correct conformation and subsequently not being able to perform their reactions; increase of wort specific gravity due to evaporation of water; the color of the wort becomes darker due to the oxidation of polyphenols and the formation of Maillard products such as melanoidins (also causes the pH to decrease), furfural and 5-HMF; boiling also stimulates the evaporation of unwanted aroma compounds (also referred to as stripping) like DMS, as previously mentioned, and desired aldehydes including Strecker aldehydes such as methional, phenylacetaldehyde, 2-methylbutanal as well as lipid oxidation aldehydes (Aron et al., 2011; Briggs et al., 2004; Fix, 1999).

In brewing process boiling is unquestionable but in whisky manufacture wort is not usually boiled to ensure that the enzyme catalyzed reactions continue to happen, which will result in higher extract yield and, as such, that more nutrients are available for the fermentation by the yeast resulting in a higher alcoholic content (Jacques et al., 2003).

1.4.6. Whirlpool and cooling

Whirlpool is a common method used in brewing to separate the hot trub and hop debris from the wort after the boil and consists of pumping the wort at high velocity into the whirlpool vessel causing the wort to start rotating resulting in the sedimentation of particles in the middle of the vessel which would otherwise be hard to remove. In order to achieve the right conditions for yeast inoculation the wort needs to be cooled. This cooling needs to be performed as fast as possible in order to reduce the risk of microbial contamination (Bamforth, 2003).

1.4.7. Fermentation

The word fermentation derives from the Latin "fevere" due to the observed effervescence caused by the release of gaseous CO_2 in alcoholic fermentation. The biological function of this process is to supply energy for yeast growth, being that part of the fermentable sugars are converted to biomass while the other part is used for the production of ethanol and carbon dioxide as well as secondary metabolites such as organic acids, longer-chain fatty acids, higher alcohols, esters, aldehydes, and other carbonyls and various sulphur containing compounds. Some of these derived products are congeners that contribute to the organoleptic profile of the beverage (Boulton, 2013; Piggott, 2015; Wanikawa, 2020).

The process is catalyzed by yeasts, predominantly, in the case of alcoholic beverages, by the species *Saccharomyces cerevisiae,* that is known for its capacity of converting sugars to ethanol and carbon dioxide at both anaerobic and aerobic conditions due to being Crabtree-positive which means that even if the oxygen is present, the yeasts will exhibit alcoholic fermentation until the levels of sugars in the growth medium are too low (Dashko et al., 2014).

The reactions that take place during fermentation may be simplified, in a gross way, by the Gay-Lussac equation:

$$C_6H_{12}O_6 \rightarrow 2\,CH_3CH_2CHO + 2\,CO_2$$

Scientifically speaking, fermentation is a metabolic process that occurs under anerobic conditions under the form of ATP (generated through substrate level phosphorylation while the redox balancing takes place mostly via the reduction of acetaldehyde to form ethanol). In the presence of oxygen, energy generation takes place via oxidative phosphorylation and redox balancing through the respiratory electron transport chain (Boulton, 2013).

Growth and metabolism are supported by the monosaccharides glucose, fructose, mannose and galactose while most strains of *S. cerevisiae* can also rely on disaccharides such as sucrose, maltose and melibiose and trisaccharide like raffinose. Substrates that are used for oxidative or respiratory growth are also available in limited supply: pyruvate, lactate, ethanol, acetate and glycerol. Acetaldehyde, a carbon compound, acts as terminal acceptor of electrons, whose generation takes place during the conversion of sugar metabolites to energy as ATP, and ethanol is formed. The usual pathway for the catabolism of sugars such as glucose, among others, is glycolysis (glycolitic pathway), the conversion of glucose to pyruvate as shown in figure 2 (Jacques et al., 2003; Scheiblauer et al., 2018).

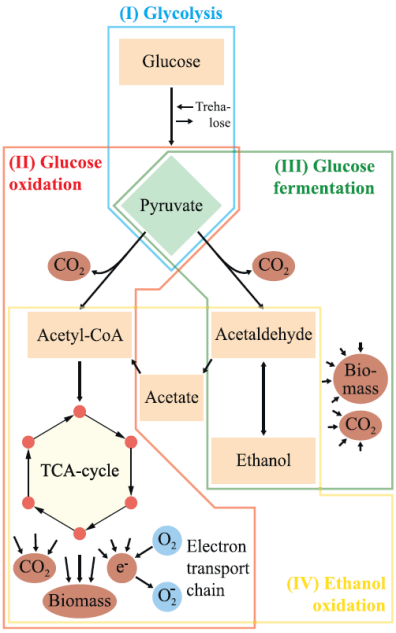

Figure 2: Main metabolic pathways during alcoholic fermentation of *Saccharomyces cerevisiae* (Scheiblauer et al, 2018)

Fermentation for alcoholic beverages by *S. cerevisiae* requires oxygen so that yeast growth is adequate as the same ensures the synthesis of lipids, unsaturated fatty acids and sterols, essential for the correct formation of the membrane structure and its functionality. These processes, in which oxygen is required, take place in the early stages of fermentation being that when oxygen is totally depleted the compounds cannot be further produced which leads to aforementioned compounds to be used up during cell growth, at which time the structure and functionality of the cell membrane is compromised and the organism growth is halted (Piggott, 2015).

The fermentation process starts when yeast is added (pitched) to the cooled and aerated wort. The early stage, lag phase, is the stage during which yeast goes from a latent phase to an active growth phase and various genes are up and down regulated, being that the oxygen levels decrease until they are no longer detectable and sterols and unsaturated fatty acids are synthesized. In the next stage (the log stage) yeasts grow exponentially, carbon dioxide is released and at the same time nutrients and sugars are absorbed

to form carbon chain skeletons to generate biomass and energy. The sugar utilization pattern is a complex process regulated by yeast in response to the presence or absence of specific sugars (at first sucrose, fructose and glucose, followed by maltose and only later long chain sugars). As it happens with sugars the uptake of nitrogen compounds is complex and regulated by a process called nitrogen catabolite repression. Many other nutrients and minerals are absorbed, such as phosphate, sulphate and many metal ions such as iron, potassium, manganese and zinc (essential co-factors in various enzymatic functions of the yeast). During the log phase the pH decreases (5-5.2 to 4) as a consequence of the flow of protons by the yeast as a result of some transport systems. When the rapid changes, characteristic of the log phase, vanish the "deceleration phase" begins, provoked by the disappearance of an essential nutrient (which may be free amino nitrogen, zinc or oxygen). This stage coincides with the absorption of all of the fermentable sugars and the start of the stationary stage (ceasing the production of CO_2 and the release of heat). The yeasts tend to separate from the wort and, in the case of some strands there is the formation of cell aggregates (flakes) in a process called flocculation and which is induced by the disappearance of fermentable sugars, being that in the case of top-fermenting strains these flakes tend to float to the surface and in the case of bottom-fermenting strains they tend to sediment. In the stationary stage yeast growth stops but the separation continues and they enter a starvation period in which they utilize the internal reservations of carbohydrates mainly glycogen, to produce energy (Boulton, 2013; Jacques et al., 2003; Piggott, 2015; Priest & Stewart, 2006).

1.4.8. Distillation

Originally the distillation of alcoholic beverages was performed by apothecaries, ecclesiastics and alchemists to produce a potable drink (*aqua ardens* or burning water) mainly for medicinal uses. Afterwards honey or sugar were added paving way to liquors. Shortly after the consumption of these beverages was done for enjoyment and thus began the steady increase in commercial distillation of spirit production (Buglass, 2011).

The distillation process consists in the application of heat for the separation of ethanol from water and other constituents in alcoholic beverages because of their boiling points, being then transferred into the distillate. The distillation of ethanol as a water-ethanol azeotropic (constant boiling point) mixture takes place at 78.5 °C along with other compounds which are more or less volatile (Mujtaba, 2004; Murray, 2014).

There are two main types of distillation apparatuses: the traditional pot still, a batch distillation with a pot still made of copper which narrows down to a vapor pipe where vapor alcohol is collected and channeled

to a water-cooled condenser causing alcohol vapor to condensate to liquid form, and column stills which are equipped with plates where the vapor is fractionated due to the reflux from the descending liquid which is taken to other plates causing it to recirculate and, thus, concentrate. Finally, the dephlegmator through the use of hot water causes compounds of higher weight to condensate leading to a more specific separation of components and increasing the ethanol content. Continuous (column) stills were invented in the early part of the 19th century and they revolutionized the distillation process by greatly increasing the yield and reducing the number of human interactions with the process, improving the purity and strength of spirits. The fact that this distillation process is characterized for being continuous and fast along with the higher concentration of alcohol in the final product are the biggest advantages over the traditional pot still and the batch column still which can only work in batches. Many different regimes and changes were implemented to give the distiller greater control over the character of the distillate such fractionators, dephlegmators, catalytic converters and ethanol (density) or temperature sensors (Berger, 2006; Karlsson & Friedman, 2017; Lee et al., 2001; Murray, 2014; Nixon & McCaw, 2001; Piggott, 2015). Both types of stills make it possible to separate different fractions or, as they are called, "cuts". The "head cut" contains volatile compounds such as acetaldehyde and ethyl acetate, while the "tail cut" contains high-boiling compounds like ethyl esters or long-chained fatty acids being that both the former and the latter contain undesirable congeners (aroma compounds) and as such are separated from the middle cut "hearts" which contain the majority of the aroma compounds pleasant for the desired organoleptic profile of the distillate. Depending on the type of distillate being produced, the choice of still to be used and the number of distillations to be performed is an integral part of the process. For example, Irish whiskey is distilled three times in a pot still while scotch whisky is distilled two times (Lee et al., 2001; Piggott, 2015).

1.4.9. Maturation

Maturation, or ageing, is recognized as one of the most important factors that influence the quality and organoleptic profile of a beverage by stimulating physicochemical interactions between wood and the liquid, that change the color and flavor and reduce both the volume and alcoholic content (Mosedale & Puech, 1998).

The traditional method to age some drinks, such as cognac or whisky, is through the use of casks which, historically, were thought to be used as storage and transportation vessel but that were revealed to be fundamental for the quality and characteristics of different products. The type and time of maturation varies a lot and frequently are defined by local or international statutes depending on the end product. There are barrels with various different volumes being that it's important to take into account that the

wood surface per liquid volume ratio will be different and, as such, a smaller barrel will have the liquid in contact with a wider wood surface area which will result in a bigger number of compounds extracted from wood. The lifespan of a barrel is limited being that it can be reused taking into account the desired characteristics of the end product. Considering that compounds will be absorbed by the wood matrix it's common to use different beverages in a barrel so that future usage will contribute to the expected final bouquet. The practice is common in wine but is growing more popular with beer and spirits. The re-use of wood is a pathway to transfer sensory properties from one beverage to another. Both sorption and absorption are connected to the enrichment of the wood with the beverage and as such, a good understanding of these processes paves the way for optimal utilization of wood as a transfer mechanism of aroma compounds for the ageing of various products (Bortoletto et al., 2016; Piggott, 2015; Spillman et al., 2004).

Oak is the most widely used wood in cooperage for barrel production (has been prevalent for about 2000 years) and maturation of beverages (like beer, wine and spirits like: whiskies, brandies, rums and liqueurs) for its properties like its permeability, handling ease, flexibility and resistance. The varieties American oak (*Quercus alba*) and French oak (*Quercus robur* and *Quercus petraea)* are highlighted. Other species have been gaining relevance for this industry, such as: *Quercus pyrenaica* and *Quercus humboldtii, Castanea sativa* (chestnut), *Robinia pseudoacacia* (acacia), *Prunus avium* (cherry), *Fraxinus excelsior* and *F.vulgaris* (ash) and *Morus alba* and *M.nigra* (mulberry) (Alañón et al., 2011; Buglass, 2011; Zhang et al., 2015).

The process of barrel manufacture starts by cutting trunks into staves or heading (for cask ends) that will be used to make the barrel, being important to note that the technique of cutting depends on the type of wood. Then, the staves pass through a process named seasoning that consists in wood drying to harden it and increase its durability that usually occurs under natural conditions in open air and so influenced by abiotic factors, process that takes usually, from 18 to 36 months. During this time degradation and oxidation phenomena take place caused by light exposure, the rain causes leaching of wood extractives and microorganisms and fungi grow in the surface releasing enzymes that breakdown wood, the humidity in wood decreases and fiber contraction occurs. The drying of wood can also occur in a kiln in which heat, humidity and air circulation are rigorously controlled. The staves now dried will undergo the process of toasting that consists in a thermal treatment in order to bend and acquire the suitable shape to the barrel construction. During this stage the degradation of wood polymers occurs generating color and flavor compounds through hydrothermolysis and pyrolysis reactions. Pyrolysis of lignin produces volatile phenols and phenolic hydroxybenzoic aldehydes, degrades polysaccharides (cellulose and hemicellulose)

produces large amounts of furaldehydes and lipid degradation results in production of aromatic aldehydes and acids such as vanillin and vanillic acid. Heat treatment is also associated with an increase of the colored compounds extracted by the spirit from wood. Three levels of toasting are defined: light, medium and heavy, regarding to the binomial heat intensity and contact time, that varies according to the type of wood. With the increase of the toasting intensity there is also an increase in quantity of volatile compounds capable of being extracted, such as furan compounds, lactones and aldehydes (Duval et al., 2013; Jacques et al., 2003; Nixon & McCaw, 2001; Piggott, 2015; Zhang et al., 2015).

Casks are often populated with microorganisms that promote significant alterations in the beverage composition, especially for products with low alcohol content. Wild yeasts such as *Brettanomyces sp*, *Debaryomyces sp* and *Candida sp*, and bacteria such us *Lactobacillus sp* and *Enterobacter sp*. The resident microbiota plays an important role in barrel aged beverages (Coelho, 2020).

Components of the unmaturated distillate may react, which is favored by its high ethanol content. During maturation a lot of reactions take place between compounds present in the distillate and in wood such as: oxidation-reductions, esterifications, polymerizations and polycondensations. The concentration of ethyl esters of fatty acids increase while esters like 3-methylbutylacetate decrease due to transesterification; aldehydes evaporate or either form acetals; volatile compounds that contribute to the aroma like “whisky lactone” or “oak lactone” (essential marker of quality of aged spirits associated with coconut and vanilla sensory descriptors), vanillin (sweet vanilla), furfural (caramel notes), guaiacol (smoky character) and eugenol (clove aroma) migrate from the wood into the distillate, giving the characteristic oak wood, smoky, vanilla-like flavor (Berger, 2006; Lee et al., 2001; Macnamara & Wyk, 2001; Pollnitz et al., 1999).

Ageing can be subdivided into subtractive and additive ageing, which will be expanded further down.

1.4.9.1. Subtractive activity

Wood is an anisotropic and porous material due to its cellular structures and orientations (where hollow lumens exist within the individual fibers, interlinked with lignin) and the liquid entry or its evaporation causes the fibers to inflate or contract, phenomena that influences the barrel permeability and subsequently the maturation process. In relation to gas exchange, particularly to oxygen, the transfer rate through wood is dependent on moisture content, being higher for dried material. Liquid evaporation creates a headspace in the top of the cask and, as a consequence, the wood that is in contact with this headspace becomes dry and so more permeable, leading to volume loss and consequently changes in internal pressure of the barrel and in the dissolved oxygen that is present in the aged beverage. Liquid

and gas exchange are responsible for the phenomena called “Angels’ share”, a result of the evaporation of the beverage that is being aged in the barrel. The constant re-use of a barrel leads to a decreased oxygen transfer rate due to the formation of precipitates in the wood’s porous structure (Buglass, 2011; Nixon & McCaw, 2001; Piggott, 2015).

The process of sorption of volatile phenolic compounds from the beverage by wood is proposed to be due to hydrophobic interactions, being that all molecules from the wood, especially lignin, can adsorb volatile phenols. Sorption also has an impact on the wood composition, which must be taken into consideration when regarding its lifecycle (Coelho et al., 2019).

All throughout the ageing process micro-oxygenation takes place, which leads to the oxidation of various compounds. This mechanism has an important part in the reduction of the volatile thiols (which have undesirable sensory descriptors); the interactions between hydrolysable tannins, copper ions and dissolved oxygen lead to active oxygen and peroxide, which in turn, degrade sulphur compounds that lead to an unwelcomed effect on the organoleptic profile, reaction with aminoacids and polymerization of phenols (which plays an important role in the pigmentation and reduction of bitterness and astringency). The interaction between aldehydes, which are frequently associated with sour and pungent descriptors, and ethanol leads to the formation of acetals which in turn are described as pleasant and fruity, contributing to the quality of the aged beverage (Berger, 2006; M. Jackson, 2004; Oberholster et al., 2015; Wanikawa, 2020).

1.4.9.2. Additive activity

One of the processes that has the biggest impact in the quality and the organoleptic profile of the beverage is the extraction of compounds from wood. These volatile compounds are extracted, initially with a very accentuated concentration increment followed by a less pronounced increase. Oak heartwood is composed by cell wall components (cellulose, hemicellulose and lignin) and low molecular weight components, the extractives (phenolic compounds, aliphatic compounds, fatty acids, lactones, terpenes, alcohols, hydrocarbons, furan compounds, steroids, norisoprenoids and inorganic substances). Inside the group of phenolics it is possible to find simple phenols (important in pigmentation, aroma and flavor) and polyphenols (natural antioxidants), that are subdivided into hydrolysable tannins (e.g., ellagitannins, important for the in-pigmentation processes and astringency of the beverage) and condensed tannins (proanthocyanidins). Oak wood adsorbs compounds from the beverages that are in contact with and so, these are then transferred into other matrices in future contacts. Depending on the beverage to be

produced, the choice between a new cask or a cask that already had contact with other matrices needs to be taken into account (Coelho, 2020; Zhang et al., 2015).

1.4.9.3. Accelerated maturation

The use of alternative solutions such as chips, staves, cubes, sticks, spirals, powder and extracts are often not only more economic but also more reliable, occupy less space in the cellar, are more eco-friendly, can be more easily combined with other innovative and alternative techniques of acceleration and allows to a finer control of the maturation due to the precise control of the wood/beverage ratio. The mentioned alternatives can be obtained from wood remnants from barrel making as well as from trees that are inappropriate for cooperage (small diameters or physical defects for example) (Oberholster et al., 2015).
The use of oak alternatives allows a further enhancement of wood compounds extraction, by modifying wood/liquid ratios and mass transfer kinetics. Wood piece size variety shows significant differences in its use, maybe due to the difference of surface area that contact with the liquid that is maturing, and in larger pieces it is possible to verify that the heat penetration is slower due to the weak coefficient of thermal conductivity. Maximum sorption capacity depends mostly on wood type rather than on the size of the wood piece (particle size influences the time needed for attaining equilibrium) (Coelho, 2020).
Micro-oxygenation is the controlled introduction of oxygen so as to mimic the slow uptake of oxygen in a barrel and has shown to have positive effects on the color intensity (increase of polymeric phenols and pigment content) and, in combination with other alternatives such as the ones described earlier, it can mimic cask maturation conditions in a much shorter length of time. Also more intensive or direct heating proposed to accelerate ageing by speeding oxidation among other reactions (Pérez-Magariño et al., 2009).
Ageing, as already mentioned, is one of the most important factors that influences the quality of distilled beverages, and is also one of the costliest ones. Wooden barrels are expensive, difficult to clean and to maintain, and so a lot of alternatives have emerged in the cooperage market in order to find a way of maintain the quality of the product reducing the impact in costs and in time regarding to the maturation phase. Legislation related to this process is very restrictive which leads to it being very difficult to implement adaptations to new methods and technologies, not to mention that the prestigious perception of traditional methods is also taken into consideration when it comes time to release a product on the market (Coelho et al., 2021; Gómez García-Carpintero et al., 2012; Power et al., 2020).

2. Materials and Methodology

2.1. Malt trials

2.1.1. Malt selection and milling

With the goal of producing a spirit similar to a single malt whisky, three base barley malts were selected: Pale Ale, Vienna and Pilsner (all of them from manufacturer Castle Malting). Using a grain mill all three malts were milled into a fine powder.

2.1.2. Mashing

Nine 250 mL Erlenmeyer flasks were used and filled with 100 mL of distilled water to which 23 g of each malt was added, thus creating three samples for each malt variety that were marked as Pa I, Pa II and Pa III, for the Pale Ale malt samples, Pi I, Pi II and Pi III, for the Pilsner Malt samples and Vi I, Vi II and Vi III for the Vienna Malt samples. The flasks were placed in a water bath at 64 °C on a tray filled with water that was heated using a water pump for circulation through a thermostat that was set to 75 °C setting the water bath temperature to 64 °C. Under this tray a magnetic agitator was placed which allowed for the rotation of a magnetic stirrer that was placed inside each individual flask. Polystyrene cubes were placed on the water to reduce heat loss from contact with air and the tray was insulated on the sides and top to keep the temperature constant during this step. The mashing process lasted 60 minutes and during this time 2 mL samples were taken each 15 minutes. The primary metabolites maltose and glucose from the samples taken at the beginning of the process (t0), after 30 minutes (t2) and at the end, after 60 min (t4) were analysed by HPLC as described in section 2.4.1.

2.1.3. Boiling and clarification

The content of the flasks was allowed to cool at room temperature and afterwards it was distributed to 50 mL falcons so that they could be centrifuged at 4000 rpm for 10 minutes. The supernatant was recovered and placed into new falcons that were placed in a water bath at 100 °C in order to boil for 30 minutes (counting from boil start). After boiling, the wort was allowed to cool down to room temperature and the content from each sample's falcons was placed in a measuring cylinder to quantify the volume of wort for each sample. Afterwards the sample was divided in two, half of which would be subjected to clarification process (centrifuged at 4000 rpm for 10 minutes, being the supernatant placed into 100 mL sterilized Erlenmeyer flasks) while the other half were placed directly into 100 mL sterilized Erlenmeyer flasks without clarification.

2.1.4. Inoculum preparation

A stock of YPD (Yeast Extract Peptone Dextrose) medium was prepared with 20 g L^{-1} of glucose, 20 g L^{-1} of peptone and 10 g L^{-1} of yeast extract and sterilized in a 250 mL Schott flask. Fermentations were conducted with a strain of *Saccharomyces cerevisiae* (Belgian Abbey Style Ale II (strain 1762) supplied by Wyeast Laboratories). Inocula were prepared by adding 20 µL of the yeast, previously taken from a stock stored in glycerol at -20 °C and allowed to defrost (stock created from permanent stock in which yeast were stored at -80 °C in a cryovial with glycerol), to 20 mL of sterile YPD media in a 100 mL Erlenmeyer flask fitted with a cotton stopper. Afterwards the Erlenmeyer flasks were placed in an incubator for yeast growth at 28 °C with orbital agitation, at a rotational speed of 180 rpm during 48 hours.

After incubation, in sterile conditions, 100 µL of the grown inoculum were transferred to a 1.5 mL Eppendorf where 900 µL of distilled water were added thus achieving a dilution factor of 10. To prepare for cell counting under the optical microscope, the Neubauer chamber was carefully cleaned with 96% alcohol. Yeast cell concentration (*C*) of the pre-inoculum was calculated resorting to the *equation 1*:

C (cell mL^{-1}) = no. of cells x (1/chamber volume (mL)) x no. of squares x dilution factor (Equation 1)

The intended yeast cellular concentration of 1 x 10^6 cells/mL, *Final Concentration* (*Cf*), and the already known cell concentration of the inoculum, *Initial Concentration* (*Ci*), allowed to calculate the volume of inoculum, *Initial Volume* (*Vi*), to be added to the wort, *Final Volume* (*Vf*), resorting to the *equation 2*

$$Ci \times Vi = Cf \times Vf \quad \text{(Equation 2)}$$

2.1.5. Pitching, Primary Fermentation and Lagering

The yeasts were pitched at the desired dilution to the Erlenmeyer flasks (fitted with rubber stoppers adapted with a fermentation airlock system filled with glycerin) with the wort and were incubated at 21 °C with orbital agitation, at a rotational speed of 150 per minute for 5 days in order to ferment (primary fermentation). During this period, twice a day, the flasks were weighed in an analytical scale to measure the system fermentation mass in order to quantify the CO_2 release to outline the fermentation profile of the different samples. After this period was over, the fermented product was decanted to separate supernatant and the trub. The volume of the supernatant of each triplicate (Pale Ale, Pale Ale clarified, Vienna, Vienna clarified, Pilsner and Pilsner clarified) was divided into two 15 mL falcons. Half of each sample was subjected to a 14-day long lagering period at 4 °C in the fridge. The primary metabolites analysed regarding to the fermentation process were glucose, maltose and ethanol present at the end of

the process by HPLC as described in section 2.4.1. The impact of the lagering process on the volatile composition was evaluated by comparing secondary metabolites differences between the samples that were and were not subjected to the process. These volatile compounds were analysed by GC-FID and GC-MS as described in section 2.4.2 and 2.4.3, respectively.

2.2. Vienna malt trials

After the analysis of both primary and secondary metabolites of the previous described trials with three different barley malts (Pale Ale, Vienna and Pilsner), the selected malt chosen to carry over the study was Vienna.

2.2.1. Milling and mashing

Using a grain mill, 640 g of Vienna malt were milled into a fine powder. Two 2 L Schott flasks were used and filled with 1 L of distilled water to which 320 g of the previously milled Vienna malt were added. The replicates were identified as Vienna 1 and Vienna 2.

The mashing for both replicates was done using an IKA C-MAG HS 7 heating plate with a magnetic agitator which allowed for the rotation of a magnetic spinner that was placed inside the shots. A perforated stopper was placed with a temperature probe for maintaining the process isothermal at 65 °C, which lasted for 60 minutes. At the end of the mashing process, a 2 mL sample from each replicate was placed in Eppendorf tubes in order to analyze primary metabolites by HPLC, and secondary metabolites by GC-FID and GC-MS as described in section 2.4.1, 2.4.2 and 2.4.3.

2.2.2. Boiling and clarification

The following process was replicated for both Vienna 1 and Vienna 2. The content of the flask with the wort was allowed to cool at ambient temperature and centrifuged at 8000 rpm for 15 min. The supernatant was recovered and placed into a new 1 L shot that was heated using a heating plate, for 30 minutes (counting from the start of the boil) at boiling temperature. After boiling, the wort was allowed to cool down to room temperature and centrifuged at 8000 rpm for 15 minutes. The clarified wort of each sample, Vienna 1 and Vienna 2, was divided in three fractions: one fraction with 800 mL placed in 1 L sterilized shot and two 50 mL fractions placed in two 100 mL sterilized Erlenmeyer flasks. The shots and the Erlenmeyer flasks were fitted with a stopper and a fermentation airlock system filled with glycerin.

2.2.3. Inoculum preparation

Two inoculums of two strains of *Saccharomyces cerevisiae* (Wyeast Belgian Abbey Style Ale II (strain 1762) and Fermentis SafAle S-33) were prepared and allowed to grow following the same steps and at the same conditions, as described in section 2.1.4. The yeast concentration of each inoculum was calculated as detailed in section 2.1.4.

2.2.4. Pitching, Primary Fermentation and Lagering

In order to evaluate the performance of the two aforementioned yeast strains, for each wort, Vienna 1 and Vienna 2, the fractions with 50 mL were inoculated each with a different yeast strain, thus resulting in two Vienna 1 samples, one inoculated with Belgian Abbey and the other with SafAle S-33, and two Vienna 2 samples, one with Belgian Abbey and the other with SafAle S-33 yeast.

Belgian Abbey yeast was pitched at the desired dilution to the two sterilized 1L shots with 800 mL of Vienna 1 and Vienna 2. The different samples were incubated at 21°C with orbital agitation, at a rotational speed of 150 per minute for 5 days in order to ferment (primary fermentation). During this period, twice a day, the shots and the flasks were weighed in an analytical scale to measure the system fermentation mass in order to quantify the CO_2 release to draw the fermentation profile of the different samples. After this period was over, the fermented products were decanted to separate supernatant and the trub. The supernatant of each was subjected to a 14-day long lagering period at 4 °C. The same primary metabolites (glucose, maltose and ethanol) were analysed for each sample after lagering

2.2.5. Distillation

The resulting products of lagering from both of the 800 mL Vienna 1 and Vienna 2 samples were distilled independently with resource to fractional distillation. The distillation system was composed of a heating mantle where the distillation flask (5 L) sat on, a Vigreux fractionation column fitted in a round bottom flask and a coolant-jacketed spiral coil condenser connected on top with a distillation head. The Vigreux column was sealed with a perforated rubber stopper through which a thermometer passed in order to control the temperature of the vapor fraction that was going up the column and thus follow the process. Also, at the end of the column, close to the joint of the distillation head a copper coil was placed to mimic copper effects of traditional pot stills. All the joints were lubricated with paraffine in order to facilitate, sealing, handling and removal. The condenser had two chambers, one, a spiral connected to an outer jacket filled with antifreeze fluid pumped from a cooled reservoir (17 °C) and other chamber where the volatile compounds condensate at the end of which they were collected in fractions of 15 mL (totaling

twelve fractions) in glass test tubes. Alongside the introduction of the fermented product in the distillation flask, glass beads were added in order to control boiling and, thus, help to refine the distillation process. From each fraction 2 mL were collected to quantify the ethanol content of each one by HPLC according to the procedure described in section 2.4.1. Then the different fractions, from one to twelve, were put together in pairs (1+2; 3+4; 5+6; 7+8; 9+10; 11+12) into 50 mL falcons and 2 mL of each pair of fractions were taken, in order to quantify secondary metabolites by GC-MS as described in section 2.4.3. After this, fractions 1 to 8 were joined together, 2 mL of the distillate were collected to an Eppendorf tube and the remaining volume was maturated with chips.

2.2.6. Induction of ageing aromas

Additive maturation of the distillates was performed using oak chips which were previously in contact with unaged fortified wine. The preparation of these final chips was performed as explained next: toasted American oak (*Quercus alba*) staves (Medium Plus toasting) from the Oenostave series (by Seguin Moreau) were cut in chips (3 x 3 x 3 mm^3), with a vertical saw in the transversal direction and with a blade in the longitudinal direction, then were submerged for two weeks in unaged fortified wine, 20.9% ABV (from Quinta do Portal S.A.) at a wood/wine ratio of 50 g L^{-1}.

The distillate products (the sum of all fractions from 1 to 8) both from Vienna 1 and Vienna 2 were placed in 100 mL flasks and subjected to ageing process with chips that were immersed in the distillates at a proportion of 20 g L^{-1}. Flasks were placed in an incubator at 40 °C, with orbital agitation at rotational speed of 150 per minute during 48 hours. After contact, the spirits were separated from the wood by decantation and 2 mL of each (Vienna 1 and Vienna 2) were put in an Eppendorf for analysis of volatile compounds by GC-MS as described in section 2.4.3.

2.2.7. Sensorial analysis

The final spirits Vienna 1 and Vienna 2 were analysed by a panel constituted by 4 experienced beer and spirits tasters in order to evaluate the sensory profile of each one. Samples were presented in tasting glasses simultaneously.

A tasting sheet with visual, aroma and taste descriptors were given to the panel, in order to proceed to the scoring using a qualitative scale from 1 to 5, being 1 the lowest correspondent qualification for the descriptor and 5 the highest score to be given (two exceptions in this scale for two visual descriptors: color (1 to 7) and legs (1 to 4)).

Geometric mean (GM) was calculated for each descriptor according to equation 3:

$$GM/\,\% = \sqrt{I \times F \times 100} \quad \text{(Equation 3)}$$

For each descriptor, the relative intensity (I) is calculated dividing the sum of the intensities given by the members of the panel for the maximum possible intensity, and the relative frequency (F) corresponds to the number of times that the descriptor was mentioned divided by the maximum number of times that it could be mentioned (Coelho et al., 2020).

2.3. Letra D and E trials

2.3.1. Distillation

Different trials took place as to evaluate the final distillate that resulted from the distillation of different beers originating from "Letra" craft brewery. "Fermentum – Engenharia das Fermentações Lda." kindly provided the beer samples: Letra D (red ale style with 6% ABV) and Letra E (Belgian dark strong ale style with 9% ABV), being the last one provided in two different versions: lagered (final product able to be commercialized) and not lagered.

The distillation system used was the same as the one described in the chapter 2.2.5 and the following adjustments were applied according to what was being studied in the different trials, all of them with 1 L of the beverage to be distilled as described next:

Letra D; Letra D with 5 g of Cascade hop in a mesh cartridge at the end of the column; Letra D with 5 g of Citra hop in a mesh cartridge at the end of the column; Letra D with 20 g of American oak chips (previously in contact with unaged fortified wine) in a mesh cartridge at the end of the column; not lagered Letra E; not lagered Letra E with 5 g of Cascade hop in a mesh cartridge at the end of the column; not lagered Letra E with 5 g of Cascade hop in a mesh cartridge at the end of the condenser; Letra E; Letra E with 5 g of Cascade hop in a mesh cartridge at the end of the column.

From the Letra D and Letra E trials, six and eight 15 mL fractions were collected, respectively. From each of them 2 mL were collected and put in Eppendorf tubes for further analysis of ethanol by HPLC as described in section 2.4.1. Afterwards fractions of each trial (1 to 6 for Letra D trials and 1 to 8 for Letra E trials) were put together in 100 mL glass flasks, from which 2 mL were taken and put on Eppendorf tubes for further analysis of volatile compounds by GC-MS as described in section 2.4.3. The remaining volume underwent through the process of maturation.

2.3.2. Maturation

The ageing process of the distillates that resulted from the different trials mentioned above was performed using oak chips, previously prepared and put in contact with unaged fortified wine, as described in section 2.2.6. After contact with wood, the spirits were separated from the chips by decantation and 2 mL of each were put in an Eppendorf tubes for analysis of volatile compounds by GC-MS as described in section 2.4.3. Considering the ethanol volume of the Letra E trials it was necessary to proceed to a dilution with distilled water to reach a final concentration of 45% ABV. Half of the volume remained as final product and the other half was again subjected to contact with wood, under the same conditions mentioned before, being sampled with the "2x" marker.

2.3.3. Sensory analysis

All the final spirits from the different trials were analysed by a panel constituted by 4 experienced alcoholic beverages tasters in order to evaluate the sensory profile of each distillate, by the same procedure described in section 2.2.7.

2.4. Analytical procedures

2.4.1. Quantification of primary metabolites by High Performance Liquid Chromatography (HPLC)

The primary metabolites were analysed by HPLC system composed by a Jasco PU-1580 pump, a Jasco AS-2057 autosampler, a Jasco RI-2031 Plus RI detector and a Knauer K-2501 UV detector at the wavelength of 210 nm. 20 µL of each sample were injected in an Aminex HPX-87H (BioRad) column, maintained at 60 °C, using 5 mM H_2SO_4 as mobile phase at a constant flow of 0.6mL/min. Chromatograms were analyzed using the Star-Chromatography Workstation software (version 6.9.3, Varian), and compounds were quantified against calibration curves prepared from pure standards.

2.4.2. Quantification of secondary metabolites by Gas Chromatography – Flame Ionization Detection

The procedure for the secondary metabolites' analysis by GC-FID was performed by direct injection of 1 µL of sample, spiked with 4-nonanol as internal standard. Samples were analyzed using a Chrompack CP-9000 gas chromatograph equipped with a split injector, a Meta-Wax (30m x 0.25 mm i.d.x 0.25 µm film thickness, Teknokroma) capillary column and a flame ion detector. Helium (Praxair) was used as carrier gas at a constant 1 mL/min flow. Injector and detector were maintained at 250 °C, and the following temperature ramp was used for the column oven: initial temperature of 60°C, maintained during 5 min followed by an increase to 177.5 °C at a 5°C/min rate and lastly by another increase to 230 °C

at 10 °C/min, which were held for an additional 15 min. Quantification was performed using Star-Chromatography Workstation software (version 6.41, Varian) using response factors from calibrations curves outlined with pure compounds in regards to the internal standard (Coelho et al., 2020).

2.4.3. Quantification of secondary metabolites by Gas Chromatography – Mass Spectrometry

The procedure for the secondary metabolites analysis by GC-MS is consisted of the following steps: each 8 mL sample was extracted with 400 µL of dichloromethane (SupraSolv for gas chromatography, Merck), after adding 4-nonanol as internal standard (3.2 µg). Extractions were performed in Pyrex tubes fitted with Teflon caps, with agitation promoted by a stir bar during 15 min. Extracts were then recovered with a glass Pasteur pipette, dehydrated with anhydrous sodium sulfate and analyzed in a Varian 3800 gas chromatograph equipped with a 1079 injector and an ion-trap mass spectrometer Varian Saturn 2000. Each 1 µL injection was made in splitless mode (30 s) in a SapiensWax MS column (30 m × 0.15 mm; 0.15 µm film thickness, Teknokroma). Carrier gas was helium 49 (Praxair) at a constant flow of 1.3 mL min-1. The detector was set to electronic impact mode with an ionization energy of 70 eV, a mass acquisition range (m/z) from 35 to 260 and a 610 ms acquisition interval. The oven temperature was initially set to 60 °C for 2 min and then raised to 234 °C at a rate of 3 °C min-1, raised again to 260 °C at 5 °C min^{-1} and finally maintained at 260 °C for 10 min. Injector temperature was set to 250 °C with a 30 mL min^{-1} split flow and transfer line was maintained at 250 °C. Compounds were identified using MS Workstation version 6.9 (Varian) software, by comparing mass spectra and retention indices with those of pure standards and quantified as 4-nonanol equivalents (Coelho et al., 2021).

3. Results and discussion

3.1. Malt trials

In order to produce a grain spirit, three different malts were chosen as potential raw materials: Pale Ale, Vienna and Pilsner. To determine which one had the best potential, fermentable sugars, ethanol and volatile compounds production were quantified. As one of the goals was to optimize the production process of a spirit beverage, other processes that are not as common in the distilling industry were introduced during the trials such as clarification and lagering.

3.1.1. Quantification of fermentable sugars

To quantify fermentable sugars that result from the hydrolysis of starch present in the different malts and which will be available for alcoholic fermentation by *Saccharomyces cerevisiae*, maltose and glucose were

profiled throughout the mashing process (Table 1). As visible in the results, the total concentration of maltose and glucose is higher in Vienna malt which theoretically will result in a higher concentration of ethanol content at the end of the alcoholic fermentation.

Table 1: Concentration (*C*) of fermentable sugars, maltose and glucose, throughout time (t) during the mashing process. Results represent the mean ± standard deviation of independent samples (n=3).

	C ($g\ L^{-1}$)								
	Pale Ale			Vienna			Pilsner		
t (min)	maltose	glucose	maltose + glucose	maltose	glucose	maltose + glucose	maltose	glucose	maltose + glucose
0	72.5 ± 3.8	8.4 ± 0.4	80.9	90.6 ± 0.4	9.4 ± 0.6	100.0	73.1 ± 0.5	9.1 ± 0.5	82.2
30	85.5 ± 5.2	9.5 ± 0.6	95.0	101.1 ± 0.9	9.7 ± 0.1	111.3	93.2 ± 0.7	10.7 ± 0.4	103.9
60	90.2 ± 6.3	10.3 ± 0.8	100.5	95.4 ± 5.6	9.6 ± 1.3	105.0	93.4 ± 3.1	11.0 ± 0.6	104.5

The concentration of the obtained fermentable sugars in this work is within the expected range of sugar content in wort, allowing the production of a fermented product with the proper ethanol concentration for distillation.

3.1.2. Quantification of ethanol concentration

The process of clarification was carried out for half of each malts' samples and the resulting products were fermented. Alcoholic fermentation of the different worts by yeast was accompanied by profiling of CO_2 production, presented in Figure 3.

As shown by the results, all samples displayed similar fermentation profiles, being noticeable an initial lag phase of at least 4.9 hours, which was followed by an exponential increase until ≈ 45.0 – 55 hours of fermentation where stationary phase was reached and maintained until the end of the process, which lasted for about 118 hours. It is possible to observe that both Vienna not clarified and Vienna clarified reached a higher concentration of released CO_2 resulting in a higher theoretical ethanol concentration expressed in alcohol by volume (ABV) in Table 2.

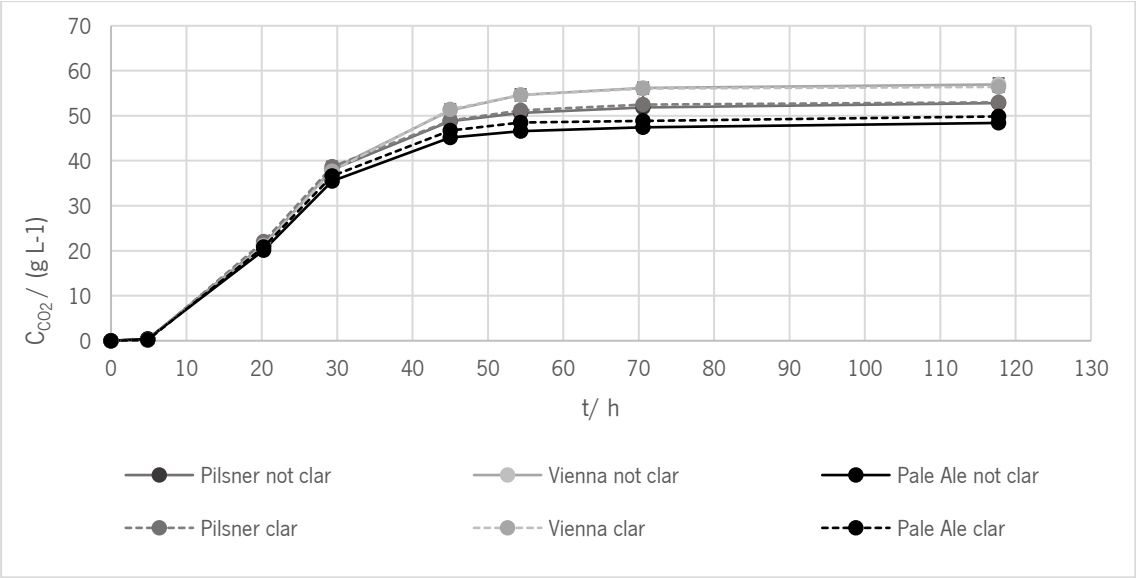

Figure 3: Profiling of CO2 production (CCO2) during alcoholic fermentation of the different samples through time (t). Results express the mean ± the standard deviation of independent samples (n=3).

The ethanol content values obtained by the profiling of CO_2 production and the quantification by HPLC are presented in Table 2.

Table 2: Alcohol by volume (ABV) regarding ethanol content determined by two quantification methods (HPLC and CO_2 production). Results represent the mean ± standard deviation of independent samples (n=3).

	ABV					
	Pale Ale		Vienna		Pilsner	
method	not clarified	clarified	not clarified	clarified	not clarified	clarified
HPLC	7.2 ± 0.3	7.0 ± 0.2	7.3 ± 0.2	7.2 ± 0.4	7.4 ± 0.5	6.9 ± 0.2
CO_2 production	6.4 ± 0.1	6.6 ± 0.3	7.6 ± 0.2	7.5 ± 0.2	7.0 ± 0.6	7.1 ± 0.1

From the previous quantification of fermentable sugars, it was expected for Vienna fermentation to achieve higher ethanol content, which was firstly verified in the profiling of CO_2 production. However, fermentation of the other malts in study presented similar ABV values in HPLC readings. Nevertheless, ethanol concentration alone is not sufficient to predict which malt is the best to serve as raw material for this study and further analysis of volatile compounds provided additional data to determine which one should be selected. Half of the samples underwent a secondary fermentation, lagering, for the maturation of the alcoholic product.

3.1.3. Characterization of the volatile compounds

To quantify volatile compounds all samples were characterized by GC-FID (major volatiles, mg L^{-1}) and GC-MS (minor volatiles, µg L^{-1}). Several groups of compounds were observed, specifically alcohols, esters, ketones, aldehydes and fatty acids that derive from yeast fermentative metabolism. Regarding major volatile compounds, ten were quantified being the corresponding concentrations in Table 3.

Alcohols were the main major volatiles found, with 2-methyl-1-propanol, 3-methyl-1-butanol, 2,3-butanediol and 2-phenylethanol at the highest concentrations. These higher alcohols are common in distilled beverages derive from yeast metabolism, namely the catabolism of sugars and amino acids via the Ehrlich pathway or anabolic glucose pathway and are described as originating pleasant aromas such as malty and flowery. Observing the sum of higher alcohols quantified in the samples, it is possible to observe that for not clarified samples of Pale Ale, Pilsner and Vienna these values seem to be decreased by lagering.

Minor volatile compounds' concentrations, presented in Table 4, show that 3-ethoxy-1-propanol, furfuryl alcohol, methionol, isoamyl acetate, 2-phenylethyl acetate (except for pale ale not clarified), octanoic acid and propanoic acid are present in similar concentrations across all samples, diethyl succinate was detected only in samples subjected to lagering and butanoic acid is present in high concentrations but only in pre-lagering samples.

As the clarification process resulted in an increase for esters, such as 2-phenilethyl acetate which contributes with floral notes reminiscent of rose and honey, while at the same time it was possible to observe a reduction in undesirable fatty acids which include propanoic acid and octanoic acid (which contribute with sweaty and rancid aromas respectively) in the clarified samples. Lagering, in turn, led to an increase in desirable compounds namely, diethyl succinate which is characterized by a fruity aroma and a further increase to the concentration of 2-phenilethyl acetate. Furthermore, not only was it possible to observe a further decrease of octanoic acid, hexanoic acid and butanoic acid also diminished all of which are associated with unpleasant aromas of rancid and stale butter. The desirable overall decrease in fatty acid concentrations is especially steeper in the clarified Vienna post-lagering sample. The clarification and lagering processes are beneficial for the overall quality of the fermented products due to protein precipitation and reduction of unpleasant compounds such as organic sulfur compounds (like diacetyl, which causes off-flavor) that were not quantified in this work but it is an interesting topic for further analysis. Since these effects are already well established, lagered samples and compounds that are usually seen as preferable to the aroma "bouquet" such as isoamyl acetate (which is described as fruity or with banana like aroma) was taken in high consideration (Briggs et al., 2004; Jacques et al., 2003; Priest & Stewart, 2006) Furthermore, according to the product sheet (Annex I) of each of the three malts, Vienna was expected to have higher presence of fruity and malty aromas. Considering that it presented the highest ethanol concentration by the end of the alcoholic fermentation and a satisfactory relative volatile composition clarified post-lagering Vienna sample was selected.

Table 3: Concentration (C) of major volatile compounds in the different fermented samples. Results represent the mean ± standard deviation of independent samples (n=3).

	Concentration (mg L^{-1})											
	Pale Ale				Pilsner				Vienna			
	Not Clarified		Clarified		Not Clarified		Clarified		Not Clarified		Clarified	
Compounds	Pre-Lag	Post-Lag	Pre-Lag	Post-Lag	Pre-Lag	Post-Lag	Pre-Lag	Post-Lag	Pre-Lag	Post-Lag	Pre-Lag	Post-Lag
Alcohols												
1-propanol	9.6 ± 1.9	32.6 ± 18.7	32.0 ± 10.6	6.0 ± 5.0	10.6 ± 1.7	5.8 ± 3.8	17.7 ± 9.4	33.5 ± 2.8	13.3 ± 0.4	4.5 ± 2.2	-	29.1 ± 2.2
2-methyl-1-propanol	78.7 ± 13.2	38.3 ± 22.0	266.6 ± 112.8	28.8 ± 19.1	91.8 ± 6.3	74.4 ± 46.3	156.6 ± 135.7	215.5 ± 24.4	156.5 ± 33.9	69.6 ± 39.2	-	181.52 ± 46.4
2-methyl-1-butanol	30.6 ± 5.2	9.9 ± 5.6	94.5 ± 40.8	5.2 ± 7.3	33.9 ± 1.8	26.3 ± 16.4	57.8 ± 50.2	76.4 ± 7.1	49.9 ± 10.4	21.9 ± 11.9	-	62.3 ± 13.4
3-methyl-1-butanol	105.4 ± 19.3	35.7 ± 20.5	348.5 ± 148.2	41.0 ± 31.5	122.6 ± 7.9	90.1 ± 55.2	201.4 ± 168.9	260.5 ± 11.4	187.1 ± 38.1	77.6 ± 42.3	-	233.5 ± 45.0
2,3-butanediol levo	55.7 ± 8.9	23.5 ± 14.4	135.7 ± 28.8	-	96.3 ± 11.9	34.2 ± 22.8	43.3 ± 7.1	169.5 ± 31.9	29.9 ± 4.9	21.3 ± 10.5	-	151.7 ± 62.9
2,3-butanediol meso	23.3 ± 3.7	6.4 ± 4.3	72.4 ± 19.5	-	26.9 ± 6.3	9.0 ± 5.8	10.6 ± 3.5	38.3 ± 10.4	10.1 ± 3.1	6.9 ± 4.9	-	36.4 ± 13.4
2-phenylethanol	170.5 ± 24.7	54.3 ± 36.3	358.8 ± 133.4	-	236.1 ± 78.3	111.8 ± 72.3	173.4 ± 111.3	219.9 ± 27.2	176.7 ± 55.4	77.6 ± 51.4	-	196.2 ± 41.8
total (mean)	**473.8**	**200.7**			**618.2**	**351.6**	**660.8**	**1013.6**	**623.5**	**279.4**		
Esters												
ethyl acetate	13.4 ± 5.8	-	25.3 ± 7.3	-	-	-	15.3 ± 9.4	37.1 ± 9.4	-	5.2 ± 1.6	-	29.6 ± 7.4
Ketones												
acetoin	-	-	-	-	-	-	-	18.6 ± 9.5	-	-	-	15.3 ± 8.6
Aldehydes												
acetaldehyde	5.3 ± 0.9	3.2 ± 1.0	40.1 ± 36.2	-	-	-	21.9 ± 8.5	56.6 ± 11.2	-	6.8 ± 3.1	-	47.8 ± 4.6

- not detected

Table 4: Concentration (*C*) of minor volatile compounds of the different samples. Results represent the mean ± standard deviation (n=3).

	Concentration (µg L-1)											
	Pale Ale				Pilsner				Vienna			
	Not Clarified		Clarified		Not Clarified		Clarified		Not Clarified		Clarified	
	Pre-lag	Post-lag	Pre-lag	Post-lag	Pre-lag	Post-lag	Pre-lag	Post-lag	Pre-lag	Post-lag	Pre-lag	Post-lag
Alcohols												
1-hexanol	64.5 ± 7.9	56.4 ± 3.0	63.7 ± 5.5	63.6 ± 24.6	55.0 ± 9.9	52.9 ± 1.3	58.5 ± 8.5	-	118.1 ± 13.9	73.5 ± 7.2	107.8 ± 7.1	84.5 ± 16.7
3-ethoxy-1-propanol	108.9 ± 20.4	85.4 ± 5.8	90.4 ± 6.5	99.0 ± 25.7	90.9 ± 3.6	93.9 ± 8.8	79.7 ± 8.5	88.0 ± 27.6	175.1 ± 26.2	97.2 ± 40.5	154.3 ± 21.6	110.0 ± 15.4
1-heptanol	-	-	21.1 ± 5.0	-	-	-	10.9 ± 2.5	-	-	-	27.9 ± 9.9	-
furfuryl alcohol	33.1 ± 2.2	35.2 ± 8.4	34.1 ± 4.3	42.4 ± 9.9	25.7 ± 6.7	24.9 ± 9.0	24.5 ± 3.7	36.2 ± 6.5	43.6 ± 7.6	41.0 ± 11.9	36.6 ± 8.4	-
methionol	613.6 ± 68.9	460.9 ± 12.1	536.9 ± 11.2	579.6 ± 73.3	484.1 ± 69.9	518.5 ± 10.4	514.2 ± 14.9	597.9 ± 126.4	729.4 ± 94.3	435.9 ± 116.7	619.9 ± 181.6	484.5 ± 114.4
total (mean)	**820.1**	**637.9**	**746.2**	**784.6**	**655.7**	**690.2**	**687.8**	**722.1**	**1066.2**	**647.6**	**946.5**	**679**
Esters												
isobutyl acetate	-	-	-	-	-	73.1 ± 37.1	-	76.2 ± 17.6	-	62.7 ± 26.1	-	-
ethyl butyrate	-	68.0 ± 9.4	85.2 ± 19.9	70.5 ± 3.1	81.1 ± 17.2	79.3 ± 1.1	90.1 ± 20.1	68.9 ± 21.3	-	66.8 ± 19.2	110.0 ± 12.8	-
isoamyl acetate	709.3 ± 157.5	910.4 ± 162.2	1 103.8 ± 223.3	1 132.5 ± 330.8	969.8 ± 287.5	1 076.2 ± 88.4	1 381.1 ± 358.8	866.0 ± 181.1	1 126.6 ± 198.7	830.6 ± 185.9	1 380.9 ± 47.8	1 168.3 ± 469.8
ethyl hexanoate	67.2 ± 22.0	25.9 ± 2.2	46.1 ± 6.5	37.1 ± 8.8	50.2 ± 0.9	37.8 ± 5.4	55.3 ± 5.1	-	67.2 ± 8.0	31.0 ± 3.5	131.9 ± 18.4	-
ethyl lactate		44.4 ± 5.0		52.8 ± 10.4		31.8 ± 7.6		-		31.7 ± 5.4		-
ethyl octanoate	-	-	-	-	-	15.5 ± 6.8	11.6 ± 0.9	-	-	-	-	-
diethyl succinate	-	23.6 ± 1.3	-	30.8 ± 4.2	-	32.3 ± 7.6	-	40.5 ± 5.5	-	28.2 ± 5.8	-	45.5 ± 11.5
2-phenylethyl acetate	229.2 ± 15.8	875.8 ± 98.8	311.5 ± 23.3	272.8 ± 57.9	257.3 ± 60.9	280.0 ± 54.8	315.8 ± 58.8	310.7 ± 44.2	295.4 ± 14.0	227.9 ± 38.9	318.1 ± 44.4	374.5 ± 56.8
total (mean)	**1005.7**	**1948.1**	**1546.6**	**1596.5**	**1358.4**	**1626**	**1853.9**	**1362.3**	**1489.2**	**1278.9**	**1940.9**	**1588.3**
Fatty Acids												
acetic acid	134.1 ± 50.2	4 257.5 ± 3621.5	199.6 ± 115.6	1 625.2 ± 1381.4	140.0 ± 109.9	5 275.4 ± 6788.1	339.0 ± 197.1	6 797.4 ± 3692.3	403.8 ± 334.5	8 561.9 ± 10 099.6	755.2 ± 372.7	6 664.6 ± 4 405.4
propanoic acid	306.8 ± 51.5	413.5 ± 139.1	274.5 ± 16.8	385.8 ± 61.1	210.0 ± 16.3	338.8 ± 205.9	205.7 ± 17.2	506.1 ± 190.0	550.6 ± 81.1	530.9 ± 386.6	526.1 ± 97.0	554.4 ± 106.6
3-methylbutyric acid	-	-	-	418.8 ± 207.6	-	555.6 ± 566.1	-	1 087.1 ± 366.7	-	735.3 ± 654.1	-	705.2 ± 216.4
butanoic acid	222.3 ± 29.0	13.5 ± 6.3	208.0 ± 38.9	-	182.8 ± 48.1	-	202.6 ± 43.5	-	356.7 ± 71.1	-	401.9 ± 62.5	-
hexanoic acid	216.8 ± 22.4	172.6 ± 17.9	156.9 ± 11.2	239.7 ± 15.4	147.3 ± 14.4	167.5 ± 14.2	147.6 ± 16.6	180.6 ± 60.9	258.1 ± 50.5	195.7 ± 79.4	227.0 ± 33.5	1 87.8 ± 43.8
octanoic acid	395.2 ± 48.2	216.7 ± 31.9	232.6 ± 81.1	282.0 ± 78.2	337.9 ± 118.4	188.8 ± 39.4	171.1 ± 54.3	126.9 ± 32.3	469.0 ± 215.3	189.5 ± 126.0	333.0 ± 84.3	161.0 ± 44.9
total (mean)	**1275.2**	**5073.8**	**1071.6**	**2951.5**	**1018**	**6526.1**	**1066**	**8698.1**	**2037.2**	**10213.3**	**2243.2**	**8274**
Volatile phenols												
4-vinylguaiacol	99.8 ± 14.5	53.2 ± 22.7	64.0 ± 7.7	60.2 ± 11.1	44.5 ± 6.6	31.8 ± 6.9	49.6 ± 8.2	-	82.1 ± 11.8	49.8 ± 6.0	65.2 ± 16.3	34.2 ± 6.9
tyrosol	186.6 ± 34.3	34.3 ± 6.4	-	45.4 ± 6.4	19.0 ± 4.9	32.6 ± 2.3	18.8 ± 2.1	-	51.5 ± 9.3	37.7 ± 20.2	36.5 ± 12.5	36.4 ± 6.9
total (mean)	**286.4**	**87.5**	**64**	**105.6**	**63.5**	**64.4**	**68.4**	**-**	**93.9**	**87.5**	**101.7**	**70.6**

- not detected

3.2. Vienna trials

3.2.1. Quantification of fermentable sugars

In order to quantify fermentable sugars present in the replicates Vienna 1 and 2, maltose and glucose were quantified at the end of the mashing process (Figure 4). As visible in the results the total concentration of maltose and glucose is higher in Vienna 1 malt which theoretically will result in a higher concentration of ethanol content at the end of the alcoholic fermentation.

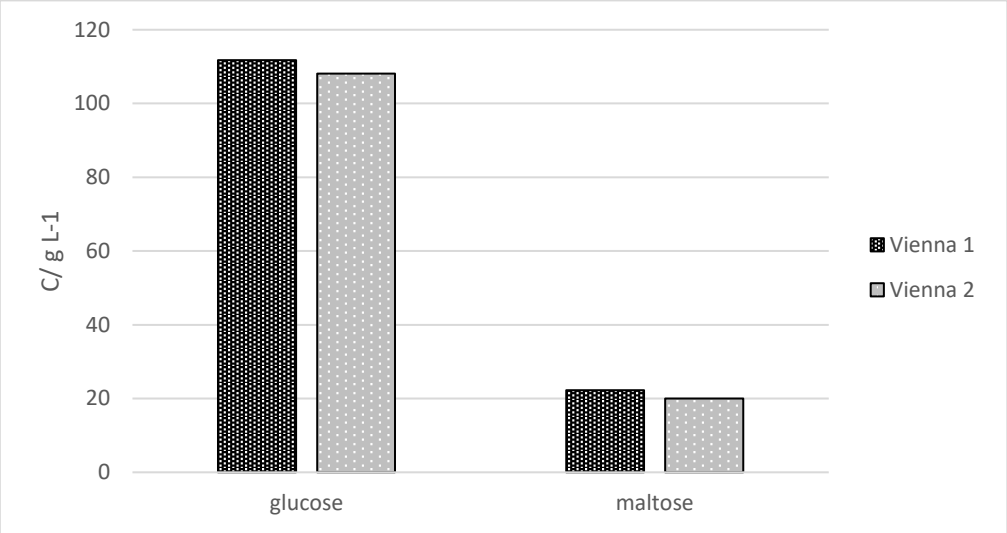

Figure 4: Concentration of glucose (*C*) and maltose at the end of the mashing process for Vienna 1 and Vienna 2 samples.

3.2.2. Quantification of ethanol concentration

The yeast strain responsible for the fermentation of all samples for malt trials was Belgian Abbey Style Ale II 1214 (BA). In order to compare the performance of the mentioned yeast strain with another strain, Fermentis SafAle S33 (s33), two fermentations were performed using Vienna 1 and Vienna 2 worts.

Alcoholic fermentation of the different worts by yeast was accompanied by profiling of CO_2 production presented in Figure 5. As shown by the results all samples displayed similar fermentation curves with a lag phase not being observed. Although s33 displayed a more vigorous start, after ≈40 hours the performance stabilized which led to it being surpassed by BA which only reached the stationary phase after ≈65 hours and were able to reach a higher CO_2 concentration.

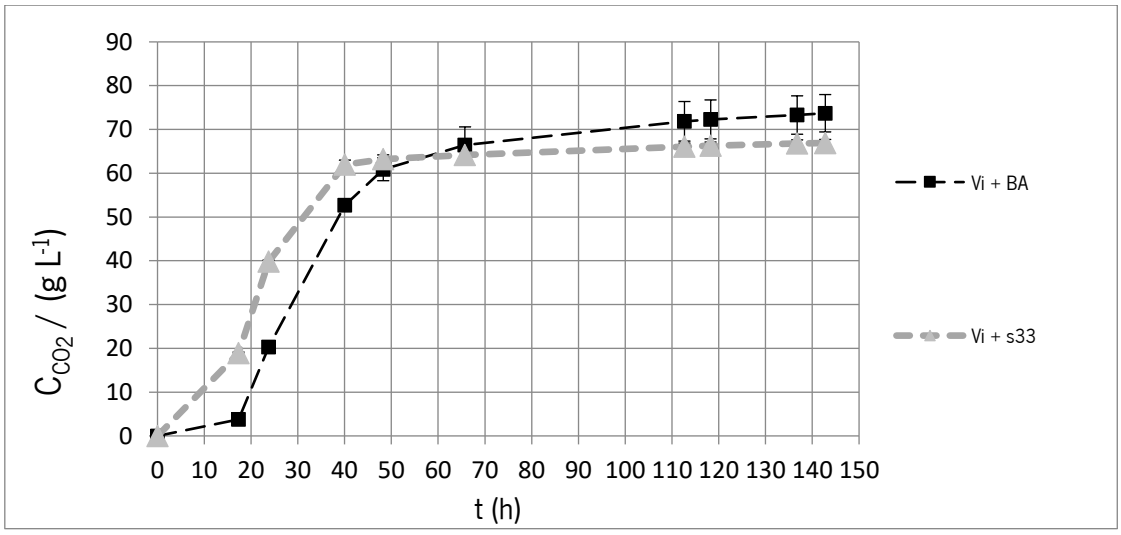

Figure 5: Profiling of CO_2 production ($C\,CO_2$) during alcoholic fermentation of the different samples through time (t). Results express the mean of Vienna 1 and Vienna 2 ± the standard deviation of independent samples (n=2).

These results were in line with the product sheet of the suppliers (Annex I and Annex II) as the BA strain is expected to reach a higher attenuation (74% - 76%) than the s33 strain (68% - 72%). The ethanol content values obtained by the profiling of CO_2 production and the quantification by HPLC are presented in Figure 6.

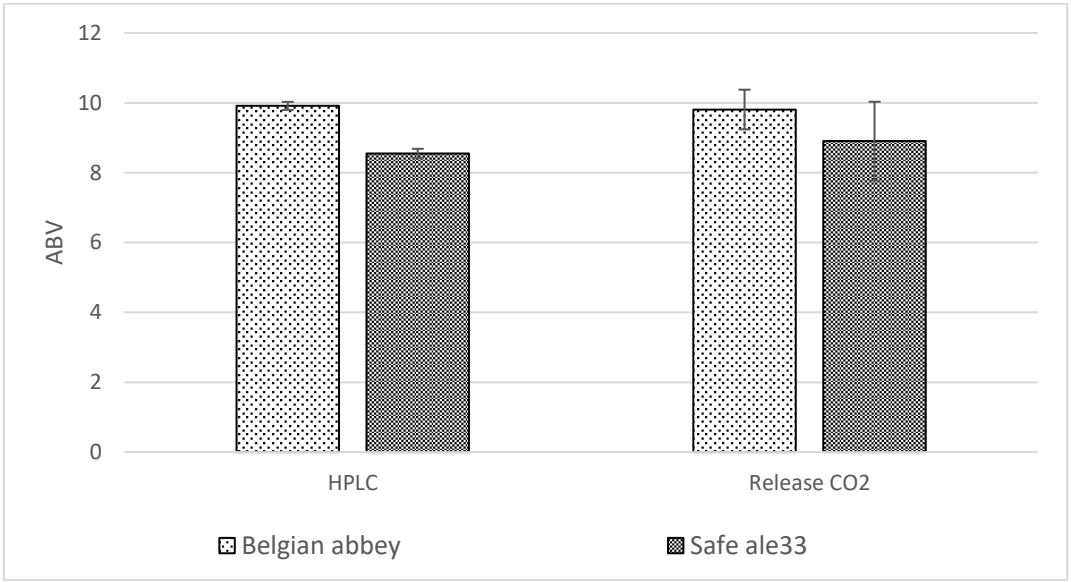

Figure 6: Alcohol by volume, regarding ethanol content derived by two methods: HPLC and CO2 profiling. Results represent the mean ± standard deviation of independent samples (n=2).

As Vienna fermented with Belgian Abbey strain obtained higher ethanol concentration, the hypothesis of this strain being a good choice for this study was confirmed, thus the wort with 0.8 L was inoculated with aforementioned strain. Afterwards, the samples underwent lagering. By analyzing the results obtained by

HPLC, Vienna 1 had as final ethanol content 9.6 ABV and Vienna 2 9.0 ABV, which is in agreement with the values of the fermentable sugars quantified also by HPLC, present in Figure 4.

After lagering both samples of Vienna 1 and Vienna 2 with Belgian Abbey were subjected to fractional distillation to increase ethanol concentration. During this process, twelve fractions of each were collected and the ethanol concentration (expressed in ABV) was measured as displayed in Figure 7.

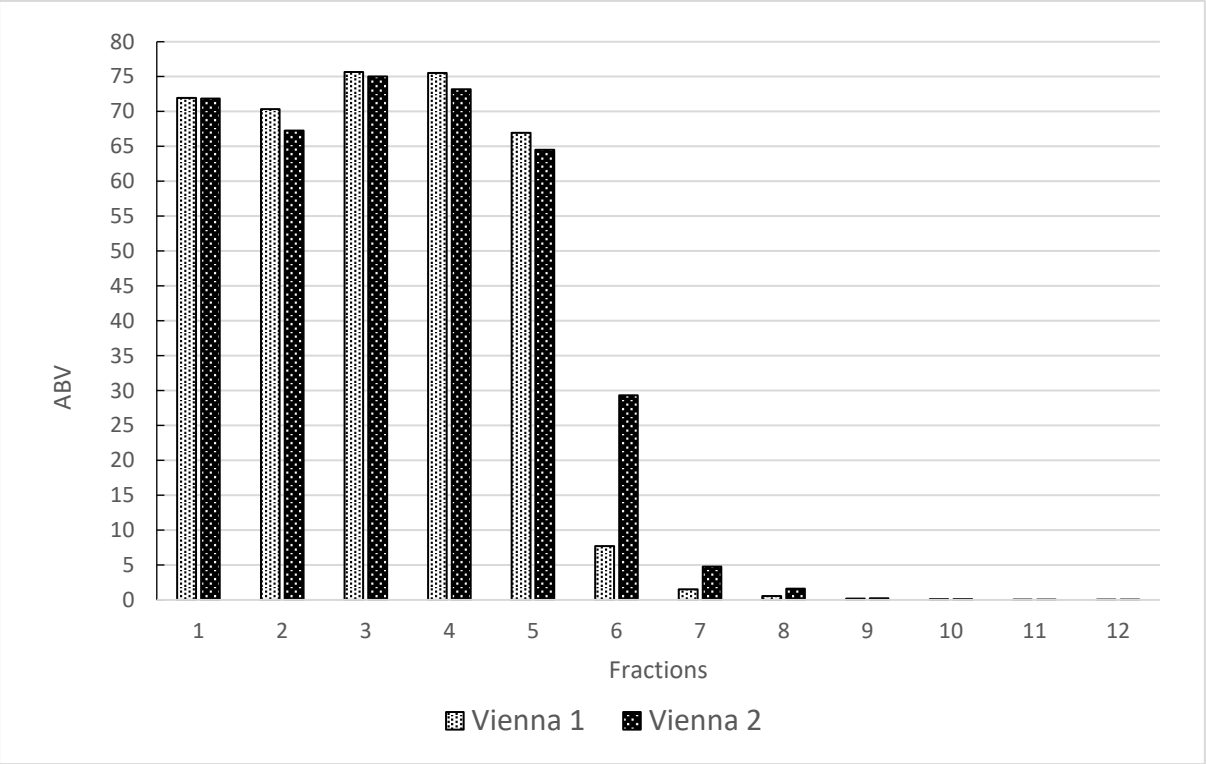

Figure 7: Profiling of ethanol concentration (ABV) in the condensed fractions collected from distillation of fermented Vienna mash.

The ethanol titter is, as expected, much higher in the first fractions with a sharp decline after the fifth fraction. Almost all ethanol was recovered in the fractions that were collected, and the final distillate obtained by mixing the fractions from 1 to 8 presented an ethanol concentration of 46.3 and 48.4 ABV for Vienna 1 and Vienna 2 respectively. These values are in agree of what it was expected for the study. The similarities between both samples demonstrates a degree of reproducibility.

3.2.2. Characterization of volatile compounds

Volatile compounds in both samples were quantified by GC-MS and the results are presented on Table 5. In general Vienna 1 and Vienna 2 concentrations of the volatile compounds were coherent between both supporting the reproducibility of the process. The fractions were analyzed in pairs (a: 1+2; b: 3+4; c: 5+6; d: 7+8; e: 9+10 and f: 11+12).

Regarding higher alcohols, 3-methyl-1-butanol presents the highest concentration of all, being present in all fractions albeit in higher concentrations in fractions a to c, showing a drastic reduction afterwards; 2-methyl-1-propanol is present in high concentrations in the first two fractions and shows a reduction in fraction c not being detected in the following fractions; methionol was only detected starting at fraction d onwards; 2-phenylethanol is present in all fractions with a marked increase in concentration starting in fraction c.

As for terpenic alcohols, linalool is present in fractions b and c as well as β-citronellol in Vienna 2 (in Vienna 1 it only appears in fraction c).

Concerning esters, isobutyl acetate is present in the first two fractions; isoamyl acetate is the ester with the highest concentration, being present in the first three fractions along with ethyl hexanoate, ethyl octanoate and ethyl decanoate; diethyl succinate is present in fractions c, d and e and ethyl phenylacetate is present in fractions b and d.

Regarding fatty acids, octanoic acid is present in fractions b to e, showing a peak in fraction c; decanoic acid appears in fraction c for Vienna 1 and in fractions b and c for Vienna 2.

The concentration of 4-vinylguaiacol decreased in Vienna 1 and Vienna 2 from fractions b to e and c to e respectively. As for the detected furan compounds, furfural appears in fraction b, shows a peak at fraction c after which it decreased until fraction e and acetyl furan decreased from fraction c to e.

The observations are coherent with what was expected and higher alcohols and esters are more plentiful in the head fractions while the tails show a higher concentration of fatty acids, furan compounds and volatile phenols (Serafim et al., 2012)

Even though traditionally the first and final fractions (respectively “hearts” and “tails”) are discarded, in this study, all fractions from 1 to 8 were mixed together to produce the final distillate because of the presence of some desirable higher alcohols and esters that are mainly present in the heads. This relation will be established by further analysis of major and minor volatile compounds.

Higher alcohols are important for the sensory profile of the distilled beverage and their presence on the first fractions demonstrates the importance of using these in the production of the distillate.

After distillation the fractions 1 through 8 of each sample were respectively mixed together and put in contact with American oak chips, and further analyzed by GC-MS to determine the impact of wood contact in the hydroalcoholic matrix. The wood contact was done by accelerated methods with the aim of producing a good quality spirit, which replicates were presented to sensory panel.

Table 5: Concentration (*C*) of minor volatile compounds of the different samples, fractions 1+2 (a), 3+4 (b), 5+6 (c), 7+8 (d), 9+10 (e), 11+12 (f). Results represent the mean ± standard deviation (n=2).

	Concentration (µg L-1)											
	Vienna 1						**Vienna 2**					
	a	b	C	d	e	f	a	b	c	d	e	f
Alcohols												
2-methyl-1-propanol	5641.3	4269.7	219.5	-	-	-	5783.6	4963.6	417.5	-	-	-
3-methyl-1-butanol	28164.1	63990.9	13194.8	40.2	22.0	22.4	33327.5	64617.6	11722.1	61.6	21.5	19.9
1-hexanol	-	74.6	44.6	-	-	-	34.4	133.9	68.6	-	-	-
1-octanol	-	62.6	89.7	-	-	-	13.9	71.1	60.4	-	-	-
methionol	-	-	-	8.3	11.1	11.9	-	-	-	11.3	7.8	11.5
2-phenylethanol	36.8	63.1	9746.9	11680.3	9965.0	9939.3	547.1	147.8	9720.4	14186.7	12596.4	12431.7
Total	**33842.2**	**68461.0**	**23295.4**	**11728.8**	**9998.1**	**9973.7**	**39706.5**	**69934.0**	**21989.1**	**14259.6**	**12625.7**	**12463.1**
Terpenic alcohols												
linalool	-	13.2	22.2	-	-	-	-	13.4	19.0	-	-	-
b-citronelol	-	-	28.2	-	-	-	-	15.0	25.3	-	-	-
geraniol	-	-	43.1	-	-	-	-	-	72.8	-	-	-
Total	**0.0**	**13.2**	**93.5**	**0.0**	**0.0**	**0.0**	**0.0**	**28.4**	**117.1**	**0.0**	**0.0**	**0.0**
Esters												
isobutyl acetate	1109.9	-	-	-	-	-	867.3	-	-	-	-	-
isoamyl acetate	22356.5	379.4	30.3	-	-	-	19866.1	460.3	25.4	-	-	-
ethyl hexanoate	497.9	32.2	33.9	-	-	-	451.1	39.4	59.4	-	-	-
ethyl octanoate	672.4	339.2	71.8	-	-	-	691.1	240.9	216.0	-	-	-
ethyl decanoate	162.0	227.3	20.0	-	-	-	167.3	127.5	59.3	-	-	-
diethyl succinate	-	-	51.4	12.9	6.4	-	-	-	63.7	17.4	7.5	-
ethyl phenylacetate	-	8.8	12.5	-	-	-	-	8.4	23.5	-	-	-
Total	**24798.7**	**986.9**	**220.0**	**12.9**	**6.4**	**0.0**	**22043.0**	**876.6**	**447.3**	**17.4**	**7.5**	**0.0**
Fatty Acids												
octanoic acid	-	33.6	5282.8	497.9	135.1	40.1	9.9	15.8	5349.3	586.0	141.3	40.8
decanoic acid	-	-	2322.8	-	-	-	-	14.9	1609.9	-	-	-
Total	**0.0**	**33.6**	**7605.6**	**497.9**	**135.1**	**40.1**	**9.9**	**30.7**	**6959.2**	**586.0**	**141.3**	**40.8**
Volatile phenols												
4-vinylguaiacol	-	-	63.4	28.5	20.1	17.7	-	-	-	26.0	19.6	17.7
Furan compounds												
furfural	-	34.4	548.5	224.0	153.5	151.4	-	47.2	545.1	302.1	221.8	213.2
2-acetylfuran	-	-	40.7	17.1	9.7	12.5	-	-	43.9	20.3	18.9	17.6
Total	**0.0**	**34.4**	**589.2**	**241.1**	**163.2**	**163.9**	**0.0**	**47.2**	**588.9**	**322.5**	**240.7**	**230.8**

- not detected

As observed in Table 6, 2-methyl-1-propanol and 3-methyl-1-butanol were the alcohols found at the highest concentrations, contributing to the organoleptic profile with malty notes; whereas 2-phenylethanol associated with the flowery descriptor is above the Perception Threshold (PT) only in Vienna 2.

Regarding terpenic alcohols, linalool was not detected after contact with wood. As for esters, ethyl octanoate and ethyl decanoate decrease after the contact with wood. Several compounds can be adsorbed from the beverage by wood, mainly due to hydrophobic interactions, impacting the final content of some alcohols, esters or acids by decreasing its concentration in the beverage.

Isoamyl acetate and 2-phenylethyl acetate were the esters with the highest concentrations, apparently not affected by contact with wood, contributing beneficially to the aroma of the final product, respectively with a banana and rose like aromas. Volatile fatty acids derive from yeast metabolism and present usually undesirable sensory defects.

The oak chips were previously in contact with wine and the compounds 3-methyl-1-butanol, 2-phenylethanol, ethyl lactate, diethyl succinate and octanoic acid are typically found in wine and, as already mentioned, the wood is a vector for compounds to transfer from a matrix to another which may explain the presence of typical compounds of wine in the distillates (Coelho et al., 2019)

Cis-oak lactone (oak wood like aroma), vanillin (vanilla like and spicy aroma) and syringaldehyde are only present after contact with wood as predicted, and have notable contribution for the sensory quality of the maturated beverage. Lactones may derive from the degradation of lipids present on wood used for cooperage, from production of the compounds by the yeast and from the transformation of the fatty acids that are present in the raw material. Aldehydes, such as vanillin and syringaldehyde are characteristic wood extractives that contributes favorably to the organoleptic profile of a beverage that undergoes the maturation process (Coelho et al., 2021)

Table 6: Concentration (*C*) of minor volatile compounds of the different samples after contact with wood and the corresponding perception thresholds (PT) reported in [15]. Results represent the mean ± standard deviation (n=2)

Compounds	PT (µg L^{-1})	Descriptors	Concentration (µg L-1)			
			Vienna 1		Vienna 2	
			Pre-wood	Post-wood	Pre-wood	Post-wood
Alcohols						
2-methyl-1-propanol	550	malty	25 420.8	31 463.4	41 693.3	37 237.0
3-methyl-1-butanol	220	malty	242 315.5	318 533.4	388 203.7	358 643.4
1-hexanol	8000	coconut; green	321.2	385.0	617.7	563.1
1-octanol	10 000	coconut; nuts; oily	362.6	344.5	295.0	303.9
2-phenylethanol	140	flowery; honey	-	100.2	-	151.6
Total			**268 420.0**	**350 826.5**	**430 809.6**	**396 899.1**
Terpenic alcohols						
linalool	25.2	Aniseed; terpene; lemon	98.0	-	121.2	-
geraniol	36	floral	-	46.0	167.5	102.4
Total			**98.0**	**46.0**	**288.7**	**102.4**
Esters						
isobutyl acetate	1 605		-	3 032.7	5 115.2	4 295.3
ethyl butyrate	20	fruity; sweet; butter	1 313.2	1 335.9	1 160.6	975.5
isoamyl acetate	30	banana; apple; solvent	50 302.8	59 940.9	59 511.4	59 661.2
ethyl hexanoate	5	fruity; green apple	1 325.4	1 642.2	1 409.5	1 527.4
ethyl lactate	157; 810	strawberry; raspberry	293.4	584.0	454.7	568.3
ethyl octanoate	5	apple; fruity; sweet	2 704.7	2 651.7	3 138.4	3 035.3
ethyl decanoate	200	fatty acid; fruity; apple; solvent	998.4	733.1	839.7	633.2
diethyl succinate	100 000		172.0	605.0	198.9	638.4
2 - phenylethyl acetate	250	rose; honey; apple; sweet	12 583.3	12 634.4	13 669.6	13 870.4
Total			**69 693.3**	**83 159.8**	**85 497.9**	**85 205.1**
Fatty Acids						
isobutyric acid	2 300	rancid butter	279.1	-	376.4	-
3-methylbutyric acid	1 500	aged cheese; feet; vomit	503.3	775.3	577.8	789.2
hexanoic acid	3 000; 8 000	vegetable oil	939.8	1 185.4	868.0	1 209.8
2-ethylhexanoic acid	3 200	bitter	1 207.5	958.2	827.5	1 042.8
octanoic acid	500	vegetable oil	114.3	129 68.6	108.5	13 716.8
decanoic acid	1 000	wax; tallow; rancid; soap	2 670.1	3120.7	1 407.4	2 252.7
undecylenic acid			-	-	392.7	745.2
Total			**5 714.2**	**19 008.3**	**4 558.3**	**19 756.4**
Volatile phenols						
2,4-di-tert-butylphenol			-	365.8	384.8	348.9
Furan compounds						
furfural	8000	smoky; almond	2 205.6	4 052.1	2 653.0	4 120.1
Lactones						
cis-oak lactone	30	oak; woody	-	654.0	-	543.0
y-nonalactone		Coconut	375.8	348.7	364.9	353.4
Total			**375.8**	**1 002.7**	**364.9**	**896.5**
Aldehydes						
vanillin	100	vanilla; spicy	-	516.0	-	493.5
syringaldehyde			-	2 386.5	-	2 630.7
Total			**0.0**	**2 902.5**	**0.0**	**3 124.1**

- not detected

3.2.3 Sensory analysis of Vienna 1 and 2

The results presented in Figure 8 and Figure 9 about the sensory analysis show the similarities both in olfactory and taste scores between the two replicas of Vienna after contact with wood, which is a good indicator of reproducibility of the production process, taking into account that replicas refer to fully independent productions. Regarding olfactory profile, the most emphasized descriptors were tree fruit, vanilla, malty and woody, all of which was predictable from the high concentrations of the volatile compounds that correspond to this aroma descriptors such as isoamyl acetate, vanillin, 2-methyl-1-propanol and 3-methyl-1-butanol and cis-oak, respectively. As for visual quality its amber color was identified in both samples, that results from the transference of phenolic compounds from the wood that modify the spirit pigmentation during ageing. The acceptance of the grain spirit with Vienna malt as raw material, fermented with Belgian Abbey yeast and aged with American oak chips previously put in contact with fortified wine, during a short period of time (48h) at 40 °C was consensual (with an overall rating of 4 out of a maximum 5) between all the panelists being this study an acceptable base for further experiments and enrichment of the knowledge the distilling industry.

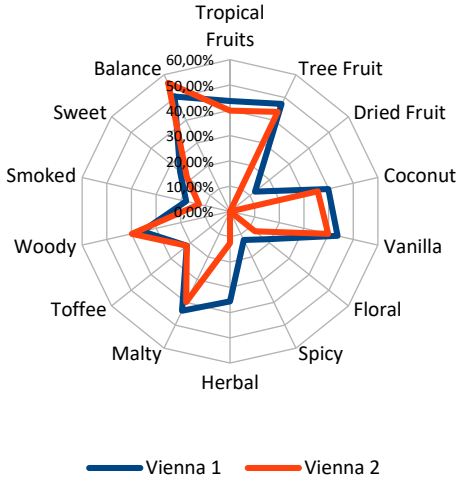

Figure 8: Aroma profile in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

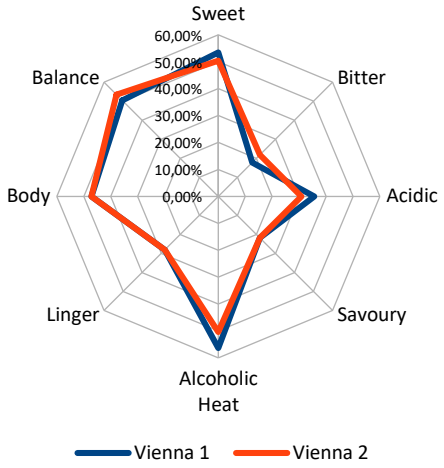

Figure 9: Taste profiles in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

3.3. Letra trials

3.3.1. Quantification of ethanol content

An exploratory approach was done in order to test if some variables introduced during the fractional distillation such as adding different hops at different locals of the distilling system would promote variances in the final distillate. Beers with commercial product designation of letra D and E (from Red Ale

and Belgian Dark Strong Ale recipes respectively) underwent fractional distillation to produce the corresponding spirits and evaluate its potential both in quality and commercial value. During this process, 6 and 8 fractions of each distillate, for letra D and letra E samples respectively, were collected and the ethanol concentration (expressed in ABV) was quantified by HPLC as displayed in Figure 10 and 11. Ethanol concentration was higher in the first three fractions with a decline after it for letra D trials, while for letra E samples the fractions from 1 to 6 present high concentrations of ethanol, being observable a decline after the last-mentioned fraction. Almost all ethanol was recovered in the fractions that were collected, and the final distillates obtained by mixing the fractions presented an ethanol concentration of around 40 % in alcohol content, although for letra E trials it was necessary to dilute the samples with purified water to meet such requirement.

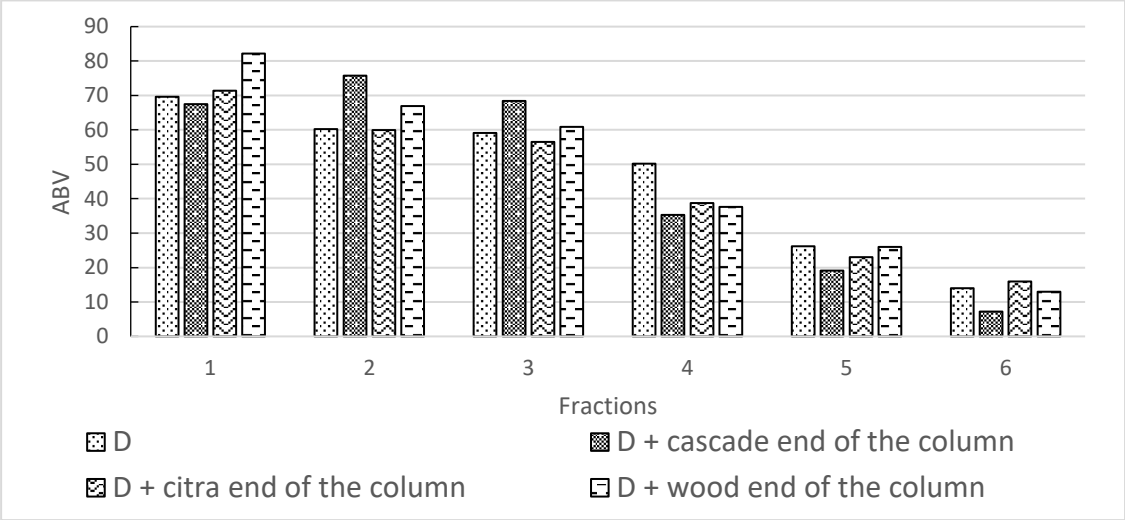

Figure 10: Profiling of ethanol concentration (ABV) in the condensed fractions collected from distillation of Letra D trials.

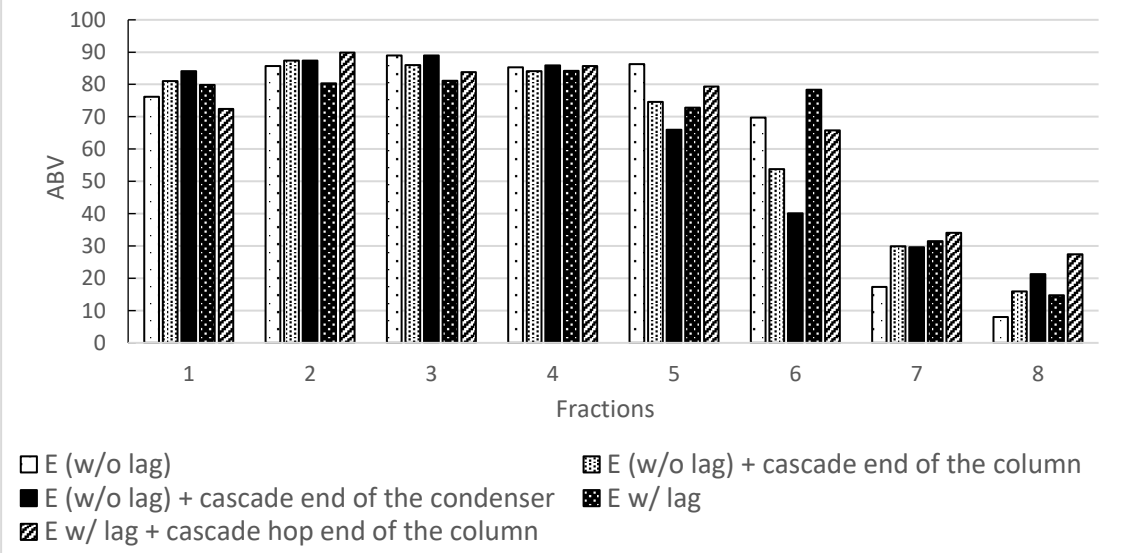

Figure 11: Profiling of ethanol concentration (ABV) in the condensed fractions collected from distillation of Letra E trials.

3.3.2. Quantification of volatile compounds

Volatile compounds of the different samples, before and after contact with oak chips, were quantified by GC-MS and the results are present in Table 7 and Table 8.

Regarding alcohols, the compounds with higher concentrations were 2-methyl-1-propanol, 3-methyl-1-butal and 2-phenylethanol. Total alcohols values were similar in all samples. Concerning terpenic alcohols, myrcene showed a higher concentration in the samples that had contact with hops (D+cascade and D+Citra); linalool and β-citronellol were the terpenes with the highest concentrations being that the sum of this group of compounds was overall higher in the samples with hop contact which is in agreement with what was expected as these are compounds that are present in high concentrations in hops. As for esters it's possible to observe that linalyl acetate was only present in the samples with hop contact, being that this compound is the acetate ester of linalool. Octanoic acid was the fatty acid that appeared in the highest concentration. *Cis*-oak lactone was present in sample D + wood before the ageing and, in all the samples that were in contact with the oak chips. Concerning aldehydes, vanillin and syringaldehyde are only present after ageing in all the samples. The mentioned aldehydes vanillin and syringaldehyde and *Cis*-oak lactone are compounds characteristically found in oak wood that were transferred to the distillate. Regarding the Letra E trials, analyzing the compounds before and after the contact with wood, in relation to alcohols it's possible to observe again the highest concentrations of 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. About terpenic alcohols it's possible to observe the presence of camphor in all the samples; myrcene was only present in the samples with hop contact both pre and post contact with wood, and for linalool, α-terpineol, β-citronellol and geraniol it was possible to observe a decrease in all samples after the ageing process. Regarding the esters, the total concentration decreases with contact with wood. *Cis*-oak lactone, vanillin and syringaldehyde are only detected after contact with wood as these compounds are characteristic of oak wood.

Table 7: Concentration (*C*) of volatile compounds of the different letra D samples before and after contact with wood quantified by GC-MS.

Compounds	Concentration (µg L-1)							
	Pre-wood				Post-wood			
	D	D + cascade end of the column	D + citra end of the column	D + wood end of the column	D	D + cascade end of the column	D + citra end of the column	D + wood end of the column
Alcohols								
2-methyl-1-propanol	14 356.2	12 217.4	13 972.7	11 147.1	12 816.8	16 217.8	10 002.5	13 518.4
3-methyl-1-butanol	103 318.0	88 056.5	86 410.0	80 228.6	93 372.0	93 445.3	69 156.4	95 580.2
4-methyl-1-pentanol	-	-	-	195.0	-	-	-	177.0
1-hexanol	566.3	570.7	536.6	586.9	559.9	585.1	534.7	604.0
2-ethyl-1-hexanol	-	-	-	129.4	-	-	-	136.0
1-octen-3-ol	191.6	244.6	245.3	197.8	245.9	299.3	263.4	195.8
1-octanol	-	108.6	114.0	143.8	-	103.7	102.1	108.1
2-phenylethanol	13 024.8	15 495.2	12 333.8	11 577.9	12 049.0	12 195.0	12 463.1	13 120.8
Total	**131 456.8**	**116 693.0**	**113 612.4**	**104 206.6**	**119 043.6**	**122 846.3**	**92 522.1**	**123 440.5**
Terpenic alcohols								
myrcene	-	1 649.6	1 155.6	330.1	-	2 658.0	750.5	195.8
linalool	2 433.8	2 386.2	2 469.9	2 464.1	2 651.4	2 569.3	2 423.3	2 451.9
a-terpineol	478.7	480.6	581.6	478.6	509.8	499.6	563.9	476.7
b-citronelol	1 561.8	1 522.0	1 624.2	1 672.1	1 757.7	1 658.7	1 672.2	1 612.2
geraniol	149.9	134.4	147.5	154.8	178.2	156.8	159.5	157.9
Total	**4 624.1**	**6 172.8**	**5 978.8**	**5 099.7**	**5 097.1**	**7 542.2**	**5 569.3**	**4 894.5**
Esters								
isoamyl acetate	889.6	483.7	727.3	679.5	790.5	601.6	555.4	656.0
ethyl hexanoate	487.2	409.6	439.6	557.7	472.7	493.3	546.9	514.9
ethyl octanoate	746.7	670.1	733.2	837.0	901.0	864.0	678.8	781.2
linalyl acetate	-	426.3	221.6	-	-	466.7	115.4	-
diethyl succinate	-	-	-	541.6	392.1	354.2	401.9	980.0
2 - phenylethyl acetate	149.0	131.4	128.4	156.1	155.7	131.2	178.6	134.9
Total	**2 272.6**	**2 121.2**	**2 250.0**	**2 771.9**	**2 712.0**	**2 911.0**	**2 477.0**	**3 066.8**
Fatty Acids								
butanoic acid	-	168.3	117.1	-	-	205.2	143.5	-
3-methylbutyric acid	134.9	-	-	120.5	163.5	-	-	195.6
octanoic acid	6 334.8	7 387.4	6 364.4	5 691.2	7 295.9	6 844.4	6 266.6	6 374.9
decanoic acid	176.2	124.1	133.6	127.0	175.4	179.2	224.0	150.3
Total	**6 645.9**	**7 679.9**	**6 615.2**	**5 938.7**	**7 634.8**	**7 228.8**	**6 634.2**	**6 720.8**
Furan compounds								
furfural	1 118.3	1 407.0	836.8	1 324.3	2 568.5	2 509.3	2 263.3	2 968.8
2-acetylfuran	668.0	750.4	475.4	534.1	638.1	622.5	548.9	570.3
5-methylfurfural	-	-	-	302.9	-	-	312.4	518.2
Total	**1 786.4**	**2 157.3**	**1 312.2**	**2 161.2**	**3 206.5**	**3 131.9**	**3 124.6**	**4 057.2**
Lactones								
cis-oak lactone	-	-	-	277.6	550.4	537.5	698.3	731.5
Aldehydes								
vanillin	-	-	-	-	388.3	579.3	959.1	518.7
syringaldehyde	-	-	-	-	2 687.2	2 447.1	8 120.8	2 800.4
Total	**0.0**	**0.0**	**0.0**	**0.0**	**3 075.5**	**3 026.4**	**9 079.9**	**3 319.0**

- not detected

Table 8: Concentration (C) of volatile compounds of the different samples of letra E before and after contact with wood quantified by GC-MS.

	Concentration (µg L-1)														
	Pre-wood					Post-wood									
Compounds	E (w/o lag)	E (w/o lag) + cascade end of the column	E (w/o lag) + cascade end of the condenser	E w/ lagering	E (w/ lag) + cascade end of the column	E (w/o lag)	E (w/o lag) **(2x)**	E (w/o lag) + cascade end of the column	E (w/o lag) + cascade end of the column **(2x)**	E (w/o lag) + cascade end of the condenser	E (w/o lag) + cascade end of the condenser **(2x)**	E w/ lagering	E w/ lagering **(2x)**	E (w/ lag) + cascade end of the column	E (w/ lag) + cascade end of the column **(2x)**
Alcohols															
2-methyl-1-propanol	35 961.7	35 330.5	40 538.0	36 880.7	40 042.5	24 767.0	23 986.7	20 657.6	19 274.1	35 785.3	18 812.7	17 347.5	14 509.6	20 658.1	21 136.8
3-methyl-1-butanol	344 620.9	440 836.0	478 056.7	492 911.0	520 311.5	247 577.7	234 723.4	230 233.6	218 151.7	381 902.6	214 918.6	246 415.6	207 129.3	266 992.9	249 527.4
4-methyl-1-pentanol	-	-	-	-	315.0	-	-	-	-	-	-	-	-	197.0	116.0
1-hexanol	311.7	458.1	508.2	417.2	463.1	216.8	248.5	248.9	244.9	378.2	274.8	238.7	222.6	226.1	255.8
1-octanol	294.3	-	-	207.0	259.3	169.4	151.7	-	-	-	-	120.9	149.7	150.9	170.6
2-phenylethanol	4 996.4	12 449.1	18 557.8	4 385.4	11 505.6	3 402.9	3 358.0	5 930.9	5 617.3	13 297.5	7 662.0	2 673.5	2 310.3	5 990.8	6 001.3
Total	**386 185.0**	**489 073.7**	**537 660.7**	**534 801.3**	**572 897.1**	**276 133.9**	**262 468.4**	**257 070.9**	**243 288.0**	**431 363.6**	**241 668.1**	**266 796.3**	**224 321.5**	**294 215.8**	**277 207.9**
Terpenic alcohols															
myrcene	-	6 266.3	1 572.5	-	7 095.6	-	-	1 934.0	2 180.6	1 906.0	617.9	-	-	2 061.5	2 656.6
camphor	1 202.0	1 353.2	1 270.6	1 310.2	1 282.9	610.8	662.6	604.8	618.7	1021.3	591.0	759.9	769.7	635.5	656.5
linalool	12 709.3	13 581.8	12 450.5	13 974.0	14 316.8	6 904.4	6 808.9	6 732.0	6 659.0	10 853.5	6 478.5	8 313.2	8 224.3	7 672.1	7 985.2
α-terpineol	652.1	860.9	756.8	558.1	622.5	315.1	293.9	360.4	363.5	582.2	328.1	315.1	306.3	313.3	410.6
β-citronelol	702.1	680.4	683.2	616.7	577.7	333.8	321.3	344.6	311.9	541.0	318.9	362.4	333.5	350.8	333.6
geraniol	206.9	301.9	262.4	159.4	-	127.9	92.9	118.4	152.0	196.1	147.3	102.8	97.8	-	-
Total	**15 472.4**	**23 044.5**	**16 996.0**	**16 618.4**	**23 895.5**	**8 291.9**	**8 179.5**	**10 094.2**	**10 285.7**	**15 100.0**	**8 481.7**	**9 853.4**	**9 731.6**	**11 033.2**	**12 042.5**
Esters															
isobutyl acetate	-	848.8	1 011.0	1 375.8	1 384.3	-	-	548.2	557.9	1 371.1	772.1	1 105.9	657.2	601.8	-
ethyl butyrate	-	1 624.4	989.4	1 428.0	-	-	-	685.7	683.6	970.6	967.9	727.3	536.0	-	-
isoamyl acetate	34 760.9	-	-	38 660.0	38 525.9	17 521.0	18 427.7	-	-	-	-	21 757.2	21 714.6	20 007.1	21 299.0
ethyl hexanoate	1673.7	1468.5	1515.5	1 484.6	1521.5	928.1	877.7	855.6	905.6	1 636.6	956.8	820.2	793.1	763.2	827.5
hexyl acetate	-	-	-	247.9	-	-	-	-	-	-	-	152.4	153.7	-	-
ethyl octanoate	4 498.2	3 125.7	2 695.6	2 402.1	2 249.9	2 160.9	2 204.6	1 651.3	1 685.9	2 935.9	1 472.8	1 200.9	1 187.4	1 118.8	1 165.3
ethyl decanoate	6 078.8	2 997.5	1 929.5	708.3	571.3	2 393.8	2 306.6	1 094.4	1 142.6	1 938.0	580.6	264.7	228.0	258.7	307.9
linalyl acetate	599.8	1 384.0	621.8	-	1 275.2	202.2	210.1	379.7	454.2	781.6	150.7	-	-	356.0	739.0
diethyl succinate	56.6	93.4	75.0	52.9	-	233.2	228.5	255.5	258.2	443.4	221.6	209.9	226.6	240.8	231.0
2 - phenylethyl acetate	5 162.4	5 809.3	4 992.0	5 867.3	5 298.8	2 699.5	2 561.1	2 615.9	2 564.5	3 817.5	2 353.1	3 355.1	3 283.3	2 761.1	2 835.0
Total	**52 830.5**	**17 351.6**	**13 829.8**	**52 227.0**	**50 826.9**	**26 138.7**	**26 816.4**	**8 086.3**	**8 252.5**	**13 894.7**	**7 475.6**	**29 593.7**	**28 779.9**	**26 107.4**	**27 404.8**
Fatty Acids															
octanoic acid	2 458.5	4 798.8	4 875.0	2 767.0	3 055.6	1 496.1	1 552.2	1 972.5	2 025.6	4 161.0	2 187.5	2 044.7	2 169.7	1 524.2	1 657.0
decanoic acid	2285.0	3251.9	2768.4	1513.3	1634.5	845.9	905.1	1230.0	1245.3	2378.4	1196.0	1018.5	1099.5	691.7	838.9
Total	**4 743.6**	**8 050.7**	**7 643.4**	**4 280.3**	**4 690.1**	**2 342.1**	**2 457.3**	**3 202.5**	**3 270.9**	**6 539.4**	**3 383.5**	**3 063.2**	**3 269.2**	**2 215.9**	**2 495.9**
Furan compounds															
furfural	909.0	813.0	774.0	678.7	752.0	1 367.2	1 282.4	1 180.0	1 038.9	1 763.1	1 025.3	1 078.8	998.2	1 131.0	1 017.6
Lactones															
cis-oak lactone	-	-	-	-	-	264.1	301.4	294.3	284.6	939.3	370.1	359.8	326.7	270.5	346.7
Aldehydes															
vanillin	-	-	-	-	-	276.9	276.0	211.8	186.3	354.6	253.5	220.8	179.4	290.2	210.3
syringaldehyde	-	-	-	-	-	1 389.3	1 320.3	1 334.9	1 369.6	2 586.9	1 519.2	1 172.5	1 144.6	1 367.1	1 478.6
Total	**-**	**-**	**-**	**-**	**-**	**1 666.2**	**1 596.2**	**1 546.6**	**1 556.0**	**2 941.5**	**1 772.6**	**1 393.2**	**1 324.0**	**1 657.2**	**1 688.8**

- not detected

3.3.3. Sensory analysis of Letra trials

All the distillates that will be analyzed in the figures are after contact with oak chips. Regarding Letra D trials it's possible to observe some differences between the samples present in the figures 12 and 13. The distillate that had as base Letra D alone was the more appreciated scoring higher on balance, sweet, tropical fruits, malty and tree fruit. It's possible to observe that D citra and D cascade have similar scores, both in the aroma, visual and taste profiles. Letra D with wood at the end of condenser has not presented higher woody and vanilla notes as expected.

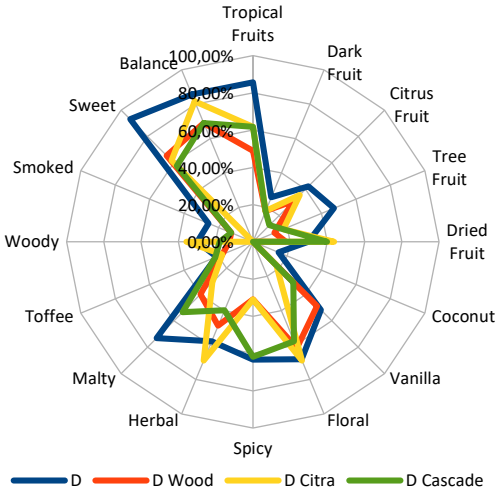

Figure 12: Aroma profile for Letra D in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

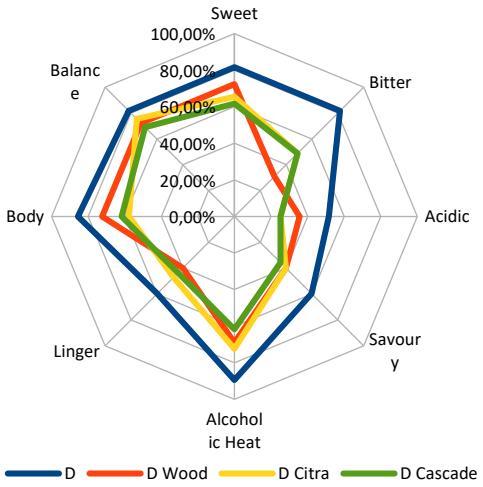

Figure 13: Taste profiles in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

As for Letra E trials, the sensory analysis was also done in the distillates after contact with American oak chips, and it is possible to observe from the figures 14 and 15 that the more appreciated, between only the lagered and unlagered samples without addition of hop in the distillation apparatus, is the distillate that had as base Letra E with lagering that was maturated twice, having higher balance, sweet and body scores taking into account the taste profile. Regarding the aroma profile, the same distillate presented higher balance, sweet, spicy and floral notes.

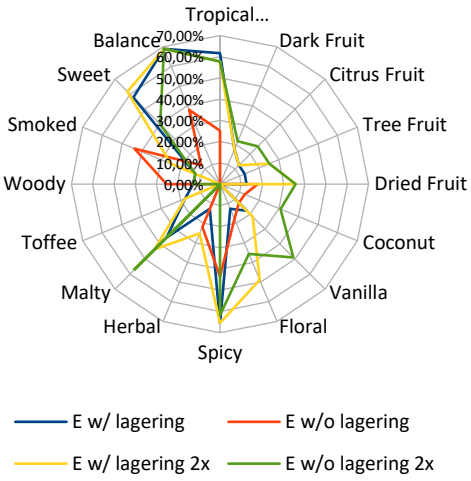

Figure 14: Aroma profile for Letra E in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

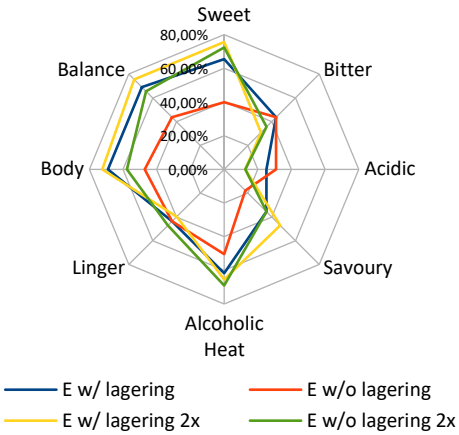

Figure 15: Taste profiles for Letra E in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

For the remaining samples with contact with cascade hop it's possible to observe in figures 16 and 17, a higher score for citrus fruit and herbal and less spicy than the ones without hop contact being the distillate that had as base Letra E with lagering put in contact with cascade hop in the condenser and twice the ageing process presents more vanilla and malty descriptors and has higher balance score both in aroma and taste profile, was for all the panelists, the most appreciated distillate produced in these trials.

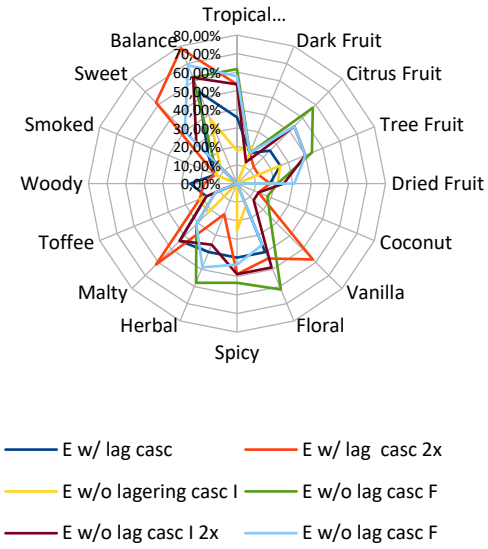

Figure 16: Aroma profile for Letra E in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

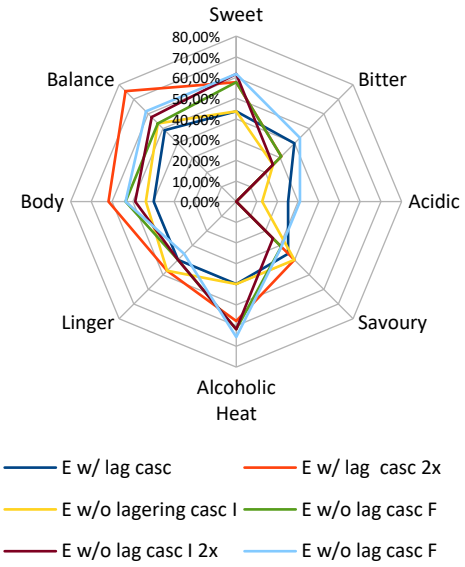

Figure 17: Taste profiles for Letra E in basis to geometric mean (GM%) of descriptors spirits maturated with oak chips.

As for visual quality the amber color was identified in most samples, that results from the transference of phenolic compounds from the wood during maturation that modify the spirit pigmentation.

4. Conclusions

The main objective of this thesis was accomplished with success as the grain spirits had high acceptance from the panelists as being similar to single malt whisky in the organoleptic profile.

The introduction of stages such as boiling, clarification and lagering had a positive impact in the final product being possible to conclude that some changes in the distillation process can be done to improve the quality of the beverages being produced.

It was possible to observe some of the differences resulting from different malts as raw-materials and chose the one with the best potential (Vienna) for the product to be made taking into account multiple parameters that were analyzed, as well as the yeast essay with two different strains, to determine which would be the most adequate, being that Belgian Abbey was confirmed to be the best choice.

The desired ethanol concentration on both the fermented product and the final distillate was achieved.

In the distillation process, the mix of all the fractions instead of the traditional separation in heads, hearts and tails revealed to be positive because some compounds associated with positive aroma descriptors were not discarded reinforcing that distilleries must pay closer attention when it comes to separate the fractions.

Multiple complimentary analysis such as the analysis of sulfur compounds, quantification of free amino nitrogen and the control of the water mineralization would result in a more profound analysis.

The trials with Letra D and Letra E confirmed that there are various changes that can be made which will result in products with good acceptance, such as the addition of hops in the distillation apparatus.

The shorter ageing periods resorting to higher temperatures of 40 °C during 48 hours with American oak chips previously submerged in fortified wine resulted in aged spirits with very good acceptance by the sensory panel being that this thesis and previous works that were used as reference, served as basis for two distillates that were produced and commercialized by FermentUM as part of Letra Craft Trials.

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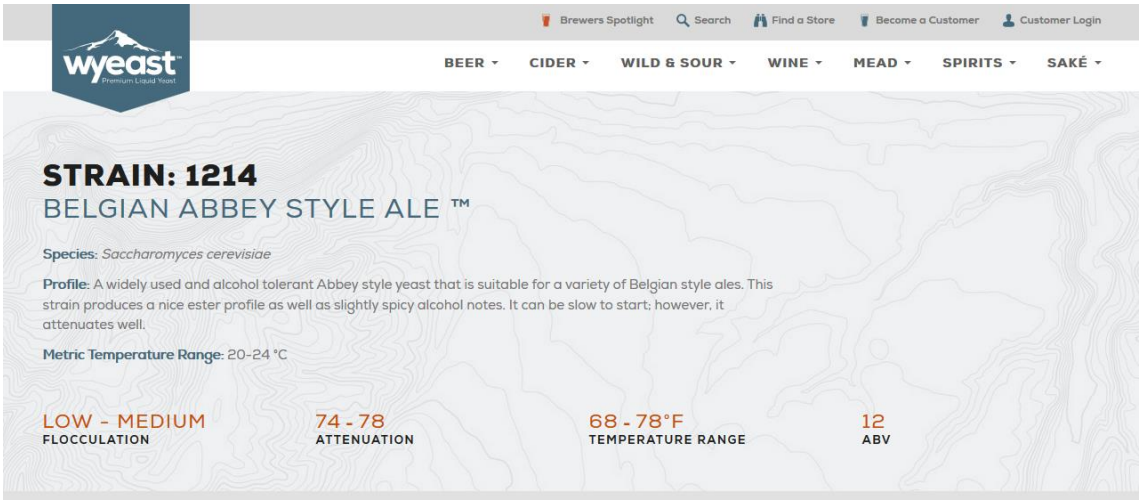
Annex I

Wyeast Strain: 1214 - Belgian Abbey Style Ale product sheet



Annex II

Fermentis SafAle S-33 Product Sheet

Fermentis by Lesaffre

E2U

SafAle™ S-33

ACTIVE DRY YEAST

IDEAL FOR SPECIAL FRUITY & HOPPED BEERS

Fruity driven strain gives a high mouthfeel and body to the beer. Ideal for Belgian Ales (Blond, Dubbel, Tripel, Quadrupel Styles) and strong English ales (ex. Imperial Stouts). It is also ideal for New England IPA's. Yeast with a medium sedimentation: forms no clumps but a powdery haze when resuspended in the beer.

Ingredients:

Yeast (*Saccharomyces cerevisiae*), emulsifier E491

Total esters	Total superior alcohols	Apparent attenuation	Flocculation	Sedimentation
medium	medium	68-72%	-	medium

Experimental conditions: standard wort in EBC tube at 18°P at 20°C.

Fermentis dry brewing yeasts are well known for their ability to produce a large variety of beer styles.
In order to compare our strains, we ran fermentation trials in laboratory conditions with a standard wort for all the strains and standard temperature conditions (SafLager: 12°C for 48h then 14°C / SafAle: 20°C). We focused on the following parameters: Alcohol production, residual sugars, flocculation and fermentation kinetic.

Given the impact of yeast of the quality of the final beer it is recommended to respect the recommended fermentation instructions. We strongly advise users to make fermentation trials before any commercial usage of our products.

Fermentation temperature: Ideally 15-20°C (59-68°F)

E2U

Pitching: Lesaffre know-how and continuous yeast production process improvement generates an **exceptional quality of dry yeasts able to resist to a very wide range of uses, incl. cold or no rehydration conditions, without affecting their viability, kinetic and/or analytical profile.** Brewers can choose usage conditions that fit the best their needs, i.e.:

With our E2U™ label, you have the choice: you can rehydrate, or you can pitch directly; depending on your equipment, habits and feelings.

- Direct Pitching

Pitch the yeast directly in the fermentation vessel on the surface of the wort at or above the fermentation temperature. Progressively sprinkle the dry yeast into the wort ensuring the yeast covers all the surface of wort available to avoid clumps. Ideally, the yeast will be added during the first part of the filling of the vessel; in which case hydration can be done at wort temperature higher than fermentation temperature, the fermenter being then filled with wort at lower temperature to bring the entire wort temperature at fermentation temperature.

- With prior rehydration

Alternatively, sprinkle the yeast in minimum 10 times its weight of sterile water or boiled and hopped wort at 25 to 29°C (77°F to 84°F). Leave to rest 15 to 30 minutes, gently stir and pitch the resultant cream into the fermentation vessel.

THE OBVIOUS CHOICE FOR BEVERAGE FERMENTATION

Fermentis division of S.I. Lesaffre • BP 3029 - 137 rue Gabriel Péri - 59703 Marcq-en-Baroeul Cedex - France • Tel.: +33(0)3 20 81 62 75 • Fax: +33(0)3 20 81 62 70 • www.fermentis.com

Annex III

Château Pale Ale malt product sheet

Belgian Malts that Make Your Beer So Special

SPECIFICATION

CHÂTEAU PALE ALE®
Crop year 2018

Parameter	Unit	Min	Max
Moisture	%		4.5
Extract (dry basis)	%	80.0	
Extract difference fine-coarse	%	1.0	2.5
Wort color	EBC(Lov.)	7.0 (3.2)	10.0 (4.3)
Total protein	%		11.5
Soluble protein	%	4.0	4.6
Kolbach index	%	38.0	45.0
Viscosity	cp		1.6
Diastatic power	WK	250	
Friability	%	80.0	
NDMA	ppb		2.5
Filtration		Normal	

Features

Belgian light-coloured base malt. Kilning at up to 90-95°C.

Characteristics

Usually used as a base malt or in combination with Pilsen 2RS malt to impart a richer malt flavour and additional colour. Being deeper in colour, this malt can add a golden hue to the wort. It is used with strong yeasts to produce amber and bitter beers. Château Pale Ale malt is kilned longer and is usually better modified, providing a more pronounced flavour than Pilsen 2RS. The enzymatic activity of Château Pale Ale malt is sufficient when used with large proportion of non-enzymatic specialty malts.

Usage

Pale ale styles and bitter beers, most traditional English beer styles. Up to 80% of the mix.

Storage and Shelf life

Malt should be stored in a clean, cool (< 22 °C) and dry (< 35 RH %) area. If these conditions are observed, we recommend to use all whole kernel products within 24 months from the date of manufacture and all milled products within 3 months.

Packaging

Bulk; Bulk in Liner Bag in Container; Bags (25kg, 50kg); Big Bags (400 - 1,400kg). All types of packaging – in 20' or 40' containers for export.

IMPORTANT

All our malts are 100% traceable from barley field through all stages of malting process up to the delivery applying and respecting Regulation EC/178/2002 of the European Council regarding traceability.

All our malts are produced using the traditional process of over 9 days, a solid warranty of high modification of the grain and real top quality of premium malts.

Annex IV

Château Vienna malt product sheet

Belgian Malts that Make Your Beer So Special

SPECIFICATION

CHÂTEAU VIENNA®
Crop year 2018

Parameter	Unit	Min	Max
Moisture	%		4.5
Extract (dry basis)	%	80.0	
Extract difference fine-coarse	%	1.5	2.5
Wort color	EBC(Lov.)	4.0 (2.1)	7.0 (3.2)
Total protein	%		11.5
Soluble protein	%	3.5	4.3
Kolbach index	%	37.0	45.0
Viscosity	cp		1.6
pH		5.6	6.0
Diastatic power	WK	250	
Friability	%	80.0	
Glassiness (whole grains)	%		2.5
Filtration		Normal	
Saccharification	Minutes	15	

Features

Belgian Vienna base malt. Lightly kilned at up to 85-90°C with shorter "cure" duration.

Characteristics

Imparts a richer flavour of malt and grain than Pilsen malt and adds subtle aromas of caramel and toffee. Château Vienna malt is kilned at slightly higher temperatures than Pilsen Malt. As a result Château Vienna malt gives a deeper golden colour to the beer increasing at the same time its body and fullness. Due to the higher kilning temperature, the enzyme activity of Château Vienna malt is slightly lower than that of Pilsen Malt. Nevertheless, our Château Vienna malt has a sufficient enzymatic activity to be used in combination with large proportion of specialty malts.

Usage

All beer styles, Vienna lager. To enhance colour and aroma of light beers. Up to 100% of the mix.

Storage and Shelf life

Malt should be stored in a clean, cool (< 22 °C) and dry (< 35 RH %) area. If these conditions are observed, we recommend to use all whole kernel products within 24 months from the date of manufacture and all milled products within 3 months.

Packaging

Bulk; Bulk in Liner Bag in Container; Bags (25kg, 50kg); Big Bags (400 - 1,400kg). All types of packaging – in 20' or 40' containers for export.

IMPORTANT

Annex V

Château Pilsen malt product sheet

Belgian Malts that Make Your Beer So Special

SPECIFICATION

CHÂTEAU PILSEN 2RS®
Crop year 2018

Parameter	Unit	Min	Max
Moisture	%		4.5
Extract (dry basis)	%	82.0	
Extract difference fine-coarse	%	1.5	2.5
Wort color	EBC(Lov.)		3.5 (1.9)
Postcoloration	EBC(Lov.)		6 (2.8)
Total protein	%		11.5
Soluble protein	%	3.5	4.4
Kolbach index	%	35.0	45.0
Viscosity	cp		1.6
Beta glucans	mg/l		220
pH		5.6	6.0
Diastatic power	WK	250	
Friability	%	80.0	
Glassiness (whole grains)	%		2.5
PDMS			5.0
Filtration		Normal	
Saccharification	Minutes		15
Clarity of wort		Clear	
Calibration: - above 2.5 mm	%	90.0	
Calibration: - rejected	%		2.0

Features

The lightest coloured Belgian malt. Produced from the finest European 2-row spring malting barley varieties. Kilned at up to 80 - 85°C.

Characteristics

The lightest in colour, this malt is well-modified and is perfectly suited for single-step infusion or for decoction mashing. Our Château Pilsen malt carries a strong, sweet malt flavour and has a sufficient enzymatic power to be used as base malt.

Usage

All beer types. Can be used up to 100 % for pale beers (Pilsner, Lager) or as part of the mix for the other beers.

Storage and Shelf life

Malt should be stored in a clean, cool (< 22 °C) and dry (< 35 RH %) area. If these conditions are observed, we recommend using all whole kernel products within 24 months from the date of manufacture and all milled