Universidade do Minho
Escola de Engenharia

Telmo Gabriel Barros Nunes

Product Development – Grape Craft Beer

Master's Degree Dissertation

Master's Degree in Food Science and Technology

Work carried out under the guidance of
Professor José Maria Marques Oliveira

October 2022

Universidade do Minho
Escola de Engenharia

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Telmo Gabriel Barros Nunes

Product Development – Grape Craft Beer

Master's Degree Dissertation

Master's Degree in Food Science and Technology

Work carried out under the guidance of
Professor José Maria Marques Oliveira

Supervisor: **João Pedro Fernandes**

October 2022

Acknowledgments

With the dissertation and internship period complete, I would first like to thank my supervisor, José Oliveira, and supervisor at the company of internship, João Fernandes, for all the help and support they gave, as well as all the patience and availability with which they answered my many doubts and questions. Furthermore, I would like to thank everyone in Sovina Lda. for the knowledge and support they provided and the open arms with which I was received. Lastly, a special thanks to my family and close friends for the never-ending support and motivation they provided, with which I could not have completed this internship.

Statement of integrity

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration. I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Product Development – Grape Craft Beer

Abstract

The craft beer market in Portugal is growing, even though it represents a small percentage of national beer production. This is a very competitive market, resulting from a growing demand for craft beer and the innovative character that is already inherent to this industry, thus putting pressure on companies to have a constant launch of new quality and outstanding products. It is therefore necessary to invest in the innovation and development of products that stand out from industrial beer, in order to draw the population's attention to this particular product and market. Innovation in this industry can be achieved by the incorporation of vinic characteristics on beer (through the use of grape must), since even though these two products share similarities on their production process, they vary dramatically in their social context, so it can be beneficial to integrate positive characteristics of wine in beer, or *vice versa*.

Through the work carried out on this study, a bottom fermentation was defined as ideal for the envisioned product, where there is a noticeable presence of the grape must used (white grape noble variety Loureiro), which was verified and confirmed by the acceptance test performed, that served as an indicator of the potential viability of this product on the market. Other characteristics such as malted and unmalted cereals, hops and guidelines for the process (such as an industrial plan and HACCP analysis) were also defined, with lager beer malts, flaked oats and citric hops conferring the best sensory profile to the final product. However, this study would beneficiate from future testing to improve the formula here used, as careful planning around the quality of the grape must and environment in which the yeast operates is essential for the final product quality.

Keywords: Craft Beer, Fermentation, Grape Ale, Grape Lager.

Desenvolvimento de Produto – Cerveja Artesanal de Uva

Resumo

O mercado da cerveja artesanal em Portugal está em crescimento, ainda que represente uma pequena percentagem da produção de cerveja nacional. Este é um mercado bastante competitivo, o que resulta de uma procura cada vez maior por cerveja artesanal e pelo caráter inovador que já é inerente a esta indústria, colocando assim pressão nas empresas para terem um lançamento constante de novos produtos de qualidade e destaque. Assim, é necessário apostar então, na inovação e desenvolvimento de produtos que se destaquem em relação à cerveja industrial, de modo a chamar a atenção da população para este produto e mercado em particular. Inovação nesta indústria pode ser conseguida através da incorporação de características vínicas no mundo da cerveja (através de mosto de uva), já que apesar de se tratarem de produtos com semelhanças ao longo do seu processo de produção, variam drasticamente no seu contexto social, daí poder ser benéfico integrar características positivas do vinho na cerveja, ou vice-versa.

Através do trabalho realizado neste estudo, definiu-se uma baixa fermentação como ideal para o produto pretendido, onde existe uma presença mais notória do mosto de uva utilizado (uva branca, casta Loureiro), o que foi verificado e confirmado pelo teste de aceitação realizado, que serviu como um indicador da potencial viabilidade deste produto no mercado. Outras características como maltes, lúpulos e diretrizes para o processo (como um plano industrial e análise HACCP) também foram definidas, com maltes de cerveja lager, aveia e lúpulos cítricos a conferirem o melhor perfil sensorial ao produto final. Contudo, este estudo beneficiaria de testes futuros para melhorar a fórmula aqui utilizada, visto que planeamento cuidadoso à volta da qualidade do mosto de uva e do ambiente no qual a levedura vai operar são essenciais para garantir a qualidade do produto final.

Palavras-chave: Cerveja Artesanal, Fermentação, Grape Ale, Grape Lager.

Table of Contents

Index of Tables

Index of Figures

1. Introduction and objectives

1.1. Objectives

This work, done under the guidance of Sovina, a craft brewery, aims to deliver a new product through the Stage-Gate model approach, which will be a strong basis for a beer that inherits characteristics from wine by the integration of grape must in the beer production process, to the market as a response to the growing need for innovation and new product development in the industry of brewing. For that, the main objective is to define the fermentation type that better suits the addition of grape must, as well as other characteristics that involve raw materials and process guidelines. In addition, innovation is necessary in several other steps of the brewing process, as different techniques of craft brewing can be applied to change the final product. Following product development it is also crucial to analyse the viability of the product in the market, through sensory analysis, as this can be interpreted as an indicator for the success or failure of this study, and adapt an HACCP and industrial plan to better ensure product quality.

This goal lines well with the context of the company where this work was carried out, as ties with Esporão imply the best of both worlds when it comes to materials and knowledge on beer and wine.

1.2. Brewery Sovina

As a company, Sovina pioneered the concept of craft beer in Portugal. The driving force behind the idea was Alberto Abreu, after several years of deepening his brewing knowledge. Alberto's passion was joined by Arménio Martins and Pedro Sousa, motivated by their common enthusiasm for beer and its brewing, forming the company "Os Três Cervejeiros" (Sovina, 2022). The project started in 2009, with a brewery store dedicated to training and providing equipment and raw materials for the production of homemade beer. Later, in 2011, after two years of preparing experimental batches and developing recipes, the first Sovina beers were produced and bottled, using only natural ingredients.

Over time, Sovina has become a reference in the premium beer sector in Portugal, contributing to the construction of a beer culture (Sovina, 2022), being known for their regular beers, the Lager, IPA, Amber, Trigo, Bock and Stout, as well as their more unique products, the 500 line of beers, which are a standard for innovation in the market.

Moreover, the company's recent connection to Esporão has given rise to a source of high-quality grapes and grape must that can be integrated in new styles of beer, making for a mix between beer and wine.

1.3. Dissertation Organization

For better understanding of the purpose and therefore, organization of this dissertation, a general overview of the contents per chapter is made here.

The current chapter, introduction mainly sets up the objectives and information on the place of internship.

On chapter 2, theoretical overview, a theoretical basis is given, approaching all matters considered relevant for this study starting on general and broader subjects narrowing on specifics for craft beer product development. The drinks industry and more specifically the beer industry are briefly talked about as to point the need and opening for this type of study on the market and on craft breweries. Also on this chapter are some theoretical foundations on development of new products, which were taken in mind when working on the various stages of this study, from planning to production. The materials for beer production are more heavily discussed in this chapter, as knowledge on the characteristics that they confer to the final product are essential on drawing the full potential of a product as complex as beer. The process behind beer production in this project is made with home brewing equipment and so, is not the focus of this study, being only succinctly mentioned.

Chapter 3, materials and methods is divided into brewing equipment and raw materials, constituting the materials part of this chapter, and, assays, sensory analysis, statistical analysis, the methods. Here, on the production process subchapter, a procedural explanation for the tests is given.

In the results and discussion, chapter 4, the statistical results of the sensory analysis assays are presented as well as a description of the resulting product of each product test. The sensory analysis evaluation was only performed after the three main product tests and therefore had no impact on the process and decision-making of product development, serving as a market study and acceptance indicators to assess the success of each product. The deciding factor at the time of product development was internal and based on the characteristics of the products. Since it was fitting to go through every stage of product development, an industrial and HACCP plan was also drawn in this chapter, based on the Sovina's factory, equipment and existing HACCP plan. These are however not the main focus of this study and are therefore subject to possible changes if the product is to be adapted to the industrial scale.

Chapter 5 contains the conclusions drawn from this study as well as future prospects for product development on this industry.

2. Theoretical Overview

2.1. Drinks Industry

The drinks industry is a complex, global network of diverse businesses that supplies most of the beverages available for consumption to the world's population. In Portugal, despite logically contributing less than the food industry to the national economy, it was still responsible for more than 17 000 direct jobs in 2020 with a total of 1967 companies, a production value of about 3000 M€ and a gross value added of 817 000 M€. When compared to the available data from 2016, where only 1793 companies existed, a positive growth trend can be observed which can be attributed to its higher export growth rate to imports in the last decade (INE, 2021). Keeping the growth level of the two streams, this industry could become a net exporter. It has maintained, in recent years, an above-average performance of the national economy, with good growth expectations in the next decade by managers and entrepreneurs in the sector. It has a high direct and indirect impact on other sectors of the Portuguese economy, with emphasis on the upstream sectors of the value chain. It assumes a great importance in entrepreneurship and job creation in less developed areas of the country.

According to the Portuguese Classification of Economic Activities, the drinks industry is a transforming industry, the division 11 of CAE rev.3, and constitutes the set of industrial activities in which beverages or ingredients for the preparation of beverages are prepared, normally in quantities that must be marketed. In 2017 this industry corresponded to approximately 4 % of the transforming industry business volume. Severall diferent industrial activities can be named that are a part of the drinks industry, with the major participants being wine production, beer production, and water and soda production. Of these, in a study conducted in 2020, wine represents 85.12 % of the number of companies, 55.11 % of business volume and 65.3 % of employed personnel. Beer on the other hand is the second largest contributor to the number of companies, business volume and jobs, with 9.67 %, 24.7 % and 14.35 % respectively (Banco de Portugal, 2021).

2.2. Drinks Industry Future

Over several years, consumers have valued food and drink products based on price, variety, attractive sensory properties and convenience. Drink industry chains have focused on consumers, who have become increasingly demanding and value nutritionally adequate, healthy and environment-friendly products at every stage of their process. As this market is so dynamic and in constant evolution, the change in the consumption of drinks has created, and will continue to have, a great influence on the

industry. With the increase in the purchasing power of consumers and the consequent increase in drinks industry products consumption, companies are betting on the development of new products (DNP) with greater added value. Globalization and the ease of communication and transport and the elimination of barriers to international trade imply a quicker spread of novelties. The industry should, therefore, continuously invest in research and development (R&D) of products with the desired characteristics, in order to guarantee their survival in the market.

2.3. Product Development

The ability to innovate is one of the biggest competitive advantages a company could have, especially when it comes to a significant change in some product, service or process, and it brings considerable benefits to the company's economy (Penso, 2003). Innovation involves new product development, production processes, marketing methods or new strategies of organization and is the result of a complex process that takes new or significantly improved ideas to the market or simply incorporates new activities essential to the innovation process (OECD, 2005).

Society, markets and products change at great speed and innovation and R&D of new products or the alteration of an existing product that can be presented to the consumer with a new name has a fundamental role in the differentiation, growth and profit of companies. The need for product development can come from an internal request (relevant legal and regulatory changes, international or national standards, codes of industrial practices, company's own needs) or an external request (customer and market needs and expectations) (OECD, 2005). New product development is a process in which the entity transforms market opportunities and technical possibilities into a commercial product, ranging from preliminary stages, such as identifying the need for this process, to final stages, such as launching and monitoring the product (Penso, 2003). However, this process requires investment in money and human resources on the part of the company and, therefore, extensive bibliographic research is needed about the product's potential, as well as planning the product's development, from its initial formulation to the final product. The objective of entities is that this process can occur as fast as possible and with the least investment, so that it becomes economically viable for the company. A lot of definitions are possible for new products, depending on their traits, however, Fuller (2016) categorizes them in 7 types, according to Table 1.

The need to increase financial profit, required by business administration, is a result of the company's growth in the market. Thus, the export of products, the implementation of marketing strategies and the constant development and introduction of new products allow the company to remain competitive and

profitable at all times. The need for new products can also be a direct result of the consumer himself, as changes in choices, lifestyle, health status, age group, education and purchasing power heavily influence market demands (Penso, 2003).

Table 1. Types of new products (adapted from Fuller, 2016)

New Products	Characteristics
Extension of a line of products	Development of a new variation of a product line. This type of product requires little research and only small changes in marketing strategy, with no need for changes to processing lines or equipment. This product also has the benefit of not requiring new raw materials, nor new storage and distribution methodology, however, it needs testing with consumers and public campaigns.
New functions for existing products	Attributing a new function (for example, health benefits) to existing products originates a type of new product, at the cost of a change in marketing and sales strategy (new label, packaging, advertising campaigns) in order to reach a new market niche.
New version of existing products	New versions of existing product, be it through changes in the process of production or new uses entirely. It involves a change of concept and for that, research and development are required. May involve changes in facilities and investment in equipment, and marketing and sales strategies require changes.
Reformulation of products	Reformulation of existing products, which could be through changes in colour, flavour, texture, appearance, caloric value, proteins, minerals, enhancing the physicochemical characteristics and/or organoleptic properties due to market needs, and reducing the cost of production of ingredients or packaging materials. The objective of this type of product is to reach new market niches and new consumption trends, requiring some research and development.
New packaging	Introduction of the same product with a new package which could aim at different goals: increasing the product's shelf life, making the packages recyclable, reducing costs, increasing practicality, adding value to the product, among others.
Innovative products	An innovative product can be the result of changes to existing products. Often requires the incorporation of new ingredients and investment in new technologies, with associated long development times and high research costs. Also requires new marketing and sales policies.
Creative product	Associated with rare products, that create a new niche in the market for themselves. They require long periods of research and development, as well as a high investment. May require completely new or even exclusive facilities and equipment. Requires an investment in marketing and sales policy. It may be necessary to create a new company or brand, and they have an associated high risk of failure as competitors are likely to quickly copy the new product at lower costs.

The life cycle of products is increasingly reduced due to market competitiveness and rapid obsolescence of products, which increases the need for new product development. Every product that is launched on the market has a life cycle that translates the acceptance of the product by the customer (expressed in sales or in generated profit) (Fuller, 2016). Initially, the product is introduced to the market and sales grow slowly and, consequently, the profit generated does not yet compensate the investments in the R&D process and in the marketing strategy. Subsequently, sales increase, as customers repeat the purchase and/or recommend to new customers, until the profit from the sales exceeds the expenses.

Eventually a slowdown in sales happens due to the appearance of new competing products and a stabilization ensues, where there is a reduction in profits and new investments are needed to change the image of the packaging and/or marketing strategies, so that the product survives. When sales drop abruptly and there is no longer any profit, the product's permanence in the market becomes unfeasible and its discontinuity may be necessary (Fuller, 2016).

For companies to survive in the market, the profit generated must last over time (Figure 1) and new products must be introduced sequentially on the market.

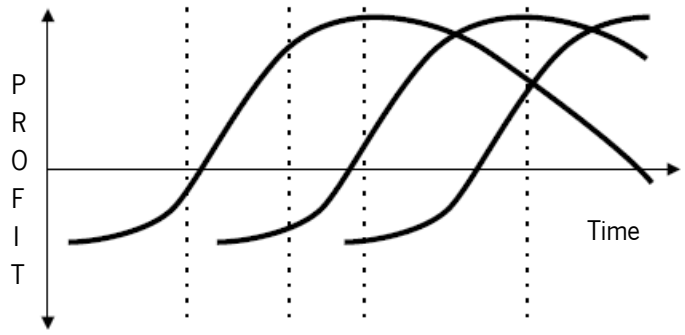

Figure 1. New product development contribution to company profit (adapted from Fuller, 2016).

New product development is challenging in the sense of the dynamism of the market, the process of understanding the expectations and desires of consumers, the details and processes that involve each step of development, the pressure of time and financial investment, and the decision-making process for launching the product. It is critical that business management ensures that this process is well managed and successful.

Due to the complexity involved in this process, it is important to assure its effectiveness and ensure the product's success in the market. For that purpose, the Stage-Gate model was hypothesized by Cooper (Cooper, 1990 and 2008), which was later adopted by several companies around the world. It is a conceptual and operational model that aims to reduce the time involved in the process of developing new products, and that intends to guarantee the good acceptability of the product on the market (sales and profit), minimizing failures and ensuring efficiency and effectiveness of the process (de Mello *et al.*, 2012). This model starts with the generation of ideas and ends with the launch of the product and consists of stages and decision "gates". At each stage, the development work is carried out where the necessary information is obtained and analysed, and with the progress of the five stages, the uncertainties and risks of the process are reduced, however, the costs increase and so does the commitment. Each step is carried out by a multidisciplinary team that covers different areas in the company (de Mello *et al.*, 2012). The decision "gates" serve as quality control, where decisions are taken in order to continue investing in

the project, and where it is verified whether the ideas are attractive from an economic and business point of view. At each decision "gate", the team wants to get results from the completed activities and fulfilled criteria (Grönlund *et al.*, 2010). The initial steps involve the generation of ideas (brainstorming) and the identification of business opportunities, and the later steps are for development, testing, validation, and commercialization, as shown in Table 2.

Table 2. Stage-Gate model (adapted from Cooper, 1990)

Step 0: Generating ideas	Generation of ideas from brainstorming, customers, complaints, R&D, competition, other markets, etc.
Gate 1	Evaluation of ideas according to strategic positioning, project feasibility, technical feasibility, magnitude of opportunity, competitive advantage, synergy with the company's main businesses and resources and market attractiveness.
Step 1: Preliminary assessment	– Preliminary investigation where size, potential and likely market acceptance are assessed; – Rapid internal assessment of the product proposal (existing technology, patents, environmental and food safety issues); – Collection of technical and market information, possible costs, and execution deadlines.
Gate 2	Project is re-evaluated with more precise criteria than gate 1, and the decision to move on to step 2 is carried out.
Step 2: Detailed evaluation	– At this stage the project must be clearly defined; – Assessment of consumer needs and desires in order to define the product to be developed; – Quantification of costs, product requirements and its market potential as well as project execution capacity (technologies and patents); – Carrying out the concept test, where the likely acceptance by the customer of the new product is determined (perceiving consumer opinions and attitudes); – Realization of the business plan: marketing plan (price, product, communication, distribution); economic-financial analysis; analysis of production and investment costs; definition of suppliers and ingredients and the distribution channel.
Gate 3	Review of each of the activities in Step 2, verifying that they were carried out and the results were positive.
Stage 3: Development	– Obtaining the product prototype (laboratory work for product development); – Product evaluation with consumers.
Gate 4	The development process is verified, ensuring that the work has been completed properly.
Step 4: Testing and validation	– Project feasibility test: the product itself; the production process; customer acceptance, and; the economic overview of the project; – Conducting product tests to verify quality and performance as well as assess customer reactions; accurately determine production costs; review the financial analysis (anticipated revenues and marketing and production costs).
Gate 5	Last chance for the project not to move forward. At this gate it is important to review the financial plans (marketing and production costs) again.
Step 5: Production and launch	The marketing and production plans are carried out, with a monitorization of the product launch. Conclusions are drawn, weighing the positives and negatives of the launch.

2.4. Sensory Analysis

Sensory analysis proves to be an essential tool that goes hand in hand with product development as a means of better perceiving the potential of a given product in the generalized view of the consumer, as well as seeing which innovation points are truly necessary or irrelevant for the quality of the in-development product. The use of sensory analysis is traced in history to wartime efforts of providing acceptable food to American forces and the development of the triangle test in Scandinavia, with a vital leap in the development of these methods being made by the Food Science Department at the University of California in 1965 (Meilgaard, Carr *et al.* 2016). Sensory analysis usage as a formalized, structured and codified methodology only came recently however, and new techniques and methods keep being developed today. Sensory testing evaluates alternative courses to select the one that optimizes value for money, and the principal uses of sensory techniques are in quality control, product development, and research. The primary function of sensory testing is to conduct valid and reliable tests that provide data on which sound decisions can be made. These methods hinge very heavily on human subjects as instruments which may prove at times difficult when the panellists are not trained in the perception required by sensory analysis (Meilgaard, Carr *et al.* 2016).

Sensory analysis tests can be divided into three main groups: discriminative or differentiation tests, affective tests, and descriptive tests. Differentiation tests are based on the perception that the taster has of the difference between products and the objective is to detect these differences, whether they are global differences or specific attributes, but does not indicate the extent of this difference (Golob, Jamnik *et al.*, 2005). These tests include for example the duo-trio test and the triangle test. Descriptive tests involve the identification and description of the different sensory attributes of a given product, by a panel of properly trained panellists (or consumers in the most recent descriptive techniques), based on the use of quantitative response scales (Murray, Delahunty *et al.*, 2001). The main descriptive tests are the simple descriptive test, sensory profile test, and free-range sensory profile test. Finally, affective tests are tests in which the consumer is asked to express his preference or acceptance for a given product, and are used in new product optimization, definition of the new product's lifespan and analysis of the potential of the new product in terms of market (comparison with competitors). These tests are the most relevant to this study and are divided into:

- Qualitative: focus group, focus panel, interviews;
- Quantitative (the preference or acceptance by the current or potential consumer of an existing or under development product is evaluated).

A part of quantitative tests are three main tests, the preference test (in which the consumer is asked to indicate the product they prefer in relation to other(s)), the acceptance test (in which the consumer is asked to indicate the degree of acceptance of one or more products) and the acceptance/expectation test (in which the consumer is asked to indicate the degree of acceptance of various products and then assess the expectation they have of the pleasantness of these products through the packaging and other elements). Preference tests are tests that only compare or rank samples among themselves, not assessing the degree of acceptance on a scale. When a sample is chosen as preferred, it does not mean that it is positively appreciated by the taster, they can all be bad but this one is the least bad. In turn, preference tests are divided into hedonic paired comparison test, hedonic multiple comparison test and hedonic ranking test. The acceptance test on the other hand is a test used to assess the degree of acceptance of one or more products by consumers in which the coded samples are presented to the taster separately (monadic form) and in a balanced order between panellists (Lawless, Heymann, 2010). The panellists then score the agreeableness of each sample on a hedonic scale (the most common is the 9-point bipolar structured discrete scale). This test provides an independent evaluation of each sample, in which the acceptance of the products can be compared with each other.

2.5. Brewing Industry

A specific sector of the drinks industry which also makes use of sensory analysis is the beer brewing industry (responsible for the production of beer). It can be said that it plays a significant role in Portugal's economy, ranking 15th among the other sectors with the highest contribution to the economy. This sector is in rapid expansion with an increase of 7 to 120 breweries from 2011 to 2017, with most being microbreweries, which are responsible for a significant disruption in the beer market with the resurgence of craft beer. In the same time period, microbreweries have increased to 115 from only 1, which amounts to 95.8 % of the breweries in operation (Machado, 2019).

Beer is consumed all over the world with a global production of 182 000 ML in 2020, which saw a decrease from previous years. In 2013, 197 000 ML of beer were produced, representing an increase of 34 % when compared to the year of 1998 (Conway, 2021a).

When it comes to the leaders in beer production, China is highlighted as the number one producer of beer, with 34 111 ML as opposed to the 21 117 ML from the United States (Conway, 2021b). Data from Portugal is only available from 2019, where 710 ML of beer were produced, which is a significantly lower amount when compared to leading countries in beer production. When compared to previous years, a

growing trend is observable since 2016, the year that marked a significant reduction of 15.7 % of the beer produced in 2011 (Conway, 2021c).

In 2020, the Belgian company AB InBev was the world's leading brewing group based on a production volume of about 46 740 ML. The company produced more than double the amount accounted for by the Dutch company, Heineken, which ranked second that year (Conway, 2021d).

In terms of its consumption, beer is the second most popular beverage on a global scale, only behind spirits, and ahead of wine (WHO, 2018). In Portugal, the beer consumption *per capita* was 51 L in 2017, which represents a low portion of the consumption in Europe (average consumption in Europe is 71 L *per capita*), falling short of countries such as the Czech Republic, Austria and Germany, which are responsible for a consumption of 138 L, 105 L and 101 L *per capita*, respectively (TBE, 2018).

2.6. Beer as a Product

As a product, beer has been around for millennia, even if the processes behind its production were not fully understood. Beer is an ensemble of three biochemical processes, the formation of enzymes on the germinating grain, the conversion of starch to sugars by these enzymes, and the fermentation of the sugars by yeasts. Its origin cannot be fully determined; however, the oldest mention of beer dates to 2800 BC in Mesopotamia, with a description of the distribution of beer and bread to a workforce. This drink was very early noticed as free of dangerous germs, which meant that water could be purified through the fermentation process. So, for many centuries, beer replaced water as the common everyday thirst-quencher (Kunze, 2004). However, the birth of a more official brewing industry came about in breweries of Christian religious foundations such as monasteries and nunneries, with beer being produced not only for personal consumption, but also as a form of payment. Its production process suffered changes throughout history and still continues to evolve today, and water, malt and hops as the only constituents of beer were defined in 1516 by the Bavarian purity law which regularized beer production from a legal standpoint, making it the first consumer protection law in the world (Kunze, 2004). Meanwhile, knowledge advanced and thanks to Louis Pasteur and his attribution of fermentation to microorganisms, new insights were now known on the fermentation of beer and the essential requirements to make it a stable product. In 1883 Emil Christian Hansen developed the single yeast propagation method (Hough, Briggs *et al.*, 1982) which was later further developed by Paul Lindner in 1893 with the drop culture method which allowed the use of pure yeast strains, diminishing contaminations and making possible the spread of light-coloured beers. Around this time marks an intensive phase of breweries being founded, with some still existing today as giant producers such as the Carlsberg breweries. During this time, other breweries

were expanding considerably with the largest producer being the Bass Brewery with an annual production of 250 000 L of beer. In the second half of the 19th century the brewing trade evolved on a global level which allowed the diffusion of knowledge on the science of brewing beer, with the creation of specialized journals, research laboratories and institutes, and brewing associations. Expert analysts also helped standardize knowledge with standard analysis methods. With this boom in the brewing industry the Bavarian purity law began to be abandoned by several countries, with the use of additives becoming increasingly popular (Kunze, 2004).

2.6.1. Raw materials

Beer is a product of four raw materials, barley, hops, water and yeast, with adjuncts being sometimes used. However, these four raw materials are the ones that influence the final quality of the product the most, hence, knowledge of their properties and effects on the process and final product are crucial (Kunze, 2004).

2.6.1.1. Barley

Barley is the main component of beer and is the source of starch required for conversion into a fermentable extract, so, a production of extract rich malts is necessary (by means of cultivation of suitable varieties). Barley is a cereal and is subdivided into two types and many varieties (Briggs, 1978), some of which may not prove very useful for beer production. These two types of barley differentiate on time of plantation, as winter barley is sown around September and spring barley around March and April. Winter and spring barleys can be subdivided in two varieties, two-row or six-row, which differ from one another on the arrangement of the corns on the ear axis (Briggs, 1978). Two-row barleys are preferably grown as spring barleys, where they possess all desirable features for malt and beer production. These barleys have larger amounts of useful contents and less husk, which translates to less polyphenolic and bitter substances. However, due to the need of this sector, the use of only one type of barley is impractical (Kunze, 2004), with two-row spring and winter, and six-row spring and winter barleys being used in the industry. Within these subclasses, barley still varies a lot in a number of different properties, with new varieties being worked on at all times, in order to improve brewing quality.

The moisture content on the grains of barley is usually 14 % to 15 % but can vary from 12 % on dry harvesting conditions, to 20 % in wet conditions, with the latter having to be dried up for storage. As for the composition of the dry weight, it consists of 70.0 % to 85.0 % of total carbohydrates, 10.5 % to 11.5 % of protein, 2.0 % to 4.0 % of inorganic matter, 1.5 % to 2.0 % of fat, with the remaining 1.0 % to 2.0 %

being attributed to other substances (Kunze, 2004). The carbohydrates present are starch, sugars, cellulose, hemicellulose and gums, and these are the most important components of barley. The starch forms 50 % to 65 % of barley and is formed by condensation of glucose, representing a form of energy reserve needed for the initial growth phase of the seedling. It is composed by two different structures, amylose and amylopectin which are glucose residues with very different structures that influence the way they are broken down in malting and mashing (Asare *et al.*, 2011). Cellulose is present on low amounts, roughly 5 % to 6 %, in the husk, where it acts as a structural component. This carbohydrate has 1,4-linked β-glucose residues, meaning that it is insoluble and cannot be broken down by malt enzymes, having no impact on the quality of the beer. Hemicellulose on the other hand do and are composed of β-glucans and pentosans. The β-glucan consist of long chains of glucose with 1,3 and 1,4 bonds and is present at about 4 % in barley. Its molecules, when in solution, form micelles as a result of formation of hydrogen bonds, so, the breakdown of these micelles constitutes a great marker of quality on beer production, and it can occur in malting or in the mashing. Pentosans consist of chains of 1,4-D-xylose residues with arabinose residues linked to it and surround β-glucan in barley having to be broken down in germination even if their impact to quality of beer is insignificant when compared to β-glucan (Kunze, 2004).

In regards of the nitrogen compounds, their content in barley can vary from 8 % to 16 %. The amount of protein in the finished beer is considered to be relatively small, with only a third of the proteins present in barley passing through the whole process. However, they still heavily influence the quality of the finished product, where they can form hazes. The proteins contained in barley are as follows: Glutelin (30 % of barley protein), Prolamin (37 % of barley protein), Globulin (15 % of barley protein, responsible for the formation of haze in beer) and Albumin (11 % of barley protein, completely precipitates when boiled) (Kunze, 2004). Since the wort is boiled, intact proteins cannot pass into the finished product, however their breakdown products can. These breakdown products are soluble in water and do not precipitate in boiling and the proportion of protein to protein breakdown products decreases during malting and brewing. These products are subdivided in high molecular weight (proteases and complex peptones which improve head retention of beer, but are also responsible for the formation of haze) and low molecular weight (amino acids and peptides) (Køie, Ingversen, Andersen, Doll, & Eggum, 1976; Klose, 2010). The amino acids are important for the yeast as they are used for the production of new cell substances (Kunze, 2004).

Lipids are also a part of the composition of barley, even if at a low percentage (2 %), and are mostly present as fatty acids. Of these, the unsaturated fatty acids take a particularly high importance, as they are known to have beneficial effects on health, but most importantly, are needed for the structure of new

yeast cells wall. Some derivatives of these fatty acids are also considered responsible for ageing processes in beer after filling, so changes in the composition of beer in fatty acids is also important (Kunze, 2004). Barley also has in its composition some mineral compounds of importance, such as phosphates, without which fermentation would not be possible. Other substances present are polyphenols and tannins, which confer harshness and bitterness (and other adverse effects such as causing haze) to the final product when present at high quantities, vitamins, which are essential for a number of metabolic processes, and finally, enzymes, one of the most important components in the process of brewing beer. These enzymes are mostly formed during germination of the barley and are necessary for the breakdown processes that occur during mashing (Willaert, 2007).

2.6.1.2. Hops

Hop refers to a perennial dioecious climbing plant of the hemp family (Moir, 2000). For brewing, only the inflorescences of the female plant are used, as these contain the bitter resins and essential oils responsible for supplying bitterness and aroma components to the beer, respectively. This plant has particular growth conditions, only being planted in special growing regions such as Germany, USA, Czech Republic and China (Kunze, 2004). After its harvest, the hops are dried (picked hops contain 75 % to 80 % water) and processed, usually into extracts or pellets, as to avoid quality reduction. Pellets are formed through the compression of dried hop milled powder and they subdivide in three types, pellets type 90, enriched pellets and isomerized pellets, each with different effects on beer quality (Bamforth, 2006).

The composition of hops is vital for the quality of the produced beer, consisting of 18.5 % bitter substances, 0.5 % hop oil, 3.5 % polyphenols, 20.0 % protein and 8.0 % minerals (Kunze, 2004). Of these, the ones that attain more importance are the bitter substances and hop oil. These bitter substances, or hop resins are initially present in the hop plant as β-acids that are only slightly bitter; however, in the course of the maturing process, these are converted into their respective α-acids which are considerably more bitter (Likens *et al.,* 1978). This is one of the factors that affects the growing conditions for desirable hops for brewing, as the conversion to α-acids only occurs at cooler temperatures and damp environments. As the type of α-acids present in the hop heavily influences the quality of the bitterness in beer, only certain varieties of hops are selected for growing. An important aspect of the bittering in beer is the isomerization of the α-acids cohumulone, humulone and adhumulone, that occurs during wort boiling according to Figure 2, and produces the respective soluble iso-α-acids (Ocvirk & Košir, 2020) which find their way to the finished beer.

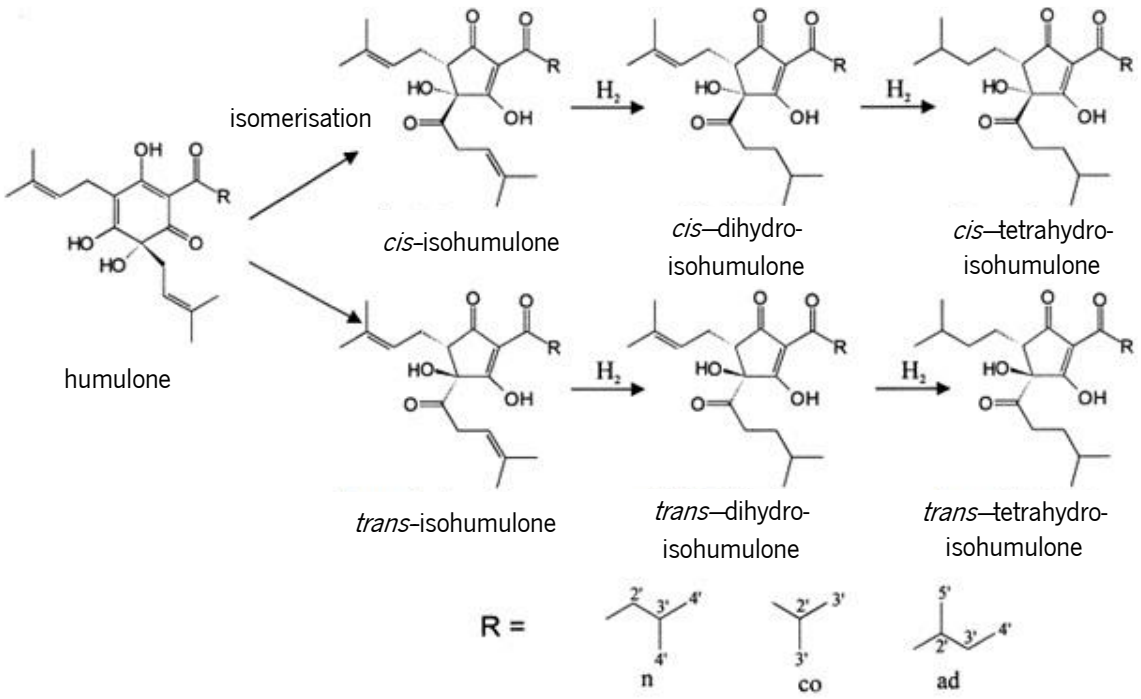

Figure 2. Isomerization of humulone (adapted from Pusecker, Albert *et al.*, 1999).

These soluble iso-α-acids are the most important bittering substances and are also surface active, improving foam stability, meaning that a better head retention occurs in strongly hopped beer. Also important is their inhibiting of the development of microorganisms in beer. Even though the three isohumulones that are a product of the mentioned isomerization of α-acids are equally bittering, the presence of cohumulone is a more interesting aspect to look at, as 36 % of the total contents of cohumulone in the hop is used for the isomerization reaction, as opposed to the 20 % and 26 % of the humulone and adhumulone contents (Meilgaard, 1960). This implies that a hop with a higher composition in cohumulone will confer an increased bitterness to the beer, as cohumulone is used preferentially (McMurrough et al., 1986). There is a lot of contradiction surrounding the bitterness conferred by cohumulone (Schönberger, 2009), but it is generally accepted to be harsh and unpleasant, attributed to the degradation of its iso-α-acids.

As iso-α-acids are not stable under thermal treatment, various degradation products form during wort boiling which can be responsible for a harsh bitterness and lingering aftertaste. This makes controlling and optimizing the boiling parameters, such as pH, original gravity and water hardness (a higher pH, lower original gravity and low water hardness reduce the usual typical losses of 25 % within 90 min of iso-α-acids), crucial for attaining a high-quality bitterness (Kappler, Krahl *et al.* 2010).

Hop oils are present at low amounts but are very important for the finished product. They are comprised of between 200 to 250 different ethereal substances which are volatile and give hops a characteristic flavour (Nance & Setzer, 2011). Their composition in hop oils is the signature of each different variety, and some of these substances could be monoterpenes (*e.g.* myrcene), diterpenes (*e.g.* dimyrcene) and sesquiterpenes (*e.g.* β-caryophyllene and humulene). As these are volatile substances, the hops meant to give aroma to the final product must be added later in the boiling process, which comes at the cost of a lower isomerization of α-acids and therefore, bitterness, or through dry hopping, adding the hops to the fermenting or maturing product to confer only aroma. Hops added for the purpose of transferring aroma to beer are considered aroma hops and their popularity has increased over the years (Kunze, 2004).

2.6.1.3. Water

Water is the major raw material in beer, quantitatively speaking, and the use of different types of water influences the quality of the beer (Hai, 2011; Punčochářová et al., 2019), with one pertaining aspect being water hardness which is influenced by calcium and magnesium dissolved in it. Breweries usually have the option of extracted ground water, spring water and surface water. One of the most important aspects of this raw material are its dissolved salts as some are required for the yeast metabolism (Birch, *et al.*, 2003), mostly present as dissociated ions as they are greatly diluted. These dissociated ions can be categorized in chemically inactive and chemically reactive, according to their capability to interact with malt components. Inactive salts affect several processes during beer production and include NaCl which gives a more "rounded" taste to beer, KCl, Na_2SO_4, K_2SO_4, etc. Reactive salts react with malt constituents during mashing and form new compounds that can affect the pH of beer, which usually is acidic, as to fasten the enzymatic processes in beer production.

2.6.1.4. Yeast

Yeasts are unicellular microorganisms which are behind the vital process of fermentation in beer. These organisms can obtain their energy either through respiration, in presence of oxygen, or through fermentation, when oxygen is absent (Rieger *et al.,* 1983). In the case of the anaerobic pathway, the wort is fermented by the yeast with a production of mainly, ethanol and CO_2. The yeasts species mainly used in beer production are *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, with selected strains systemically isolated and grown (Capece *et al.,* 2018). Knowledge of the metabolic pathways of yeast is fundamental for beer production as many products and sub-products of this microorganism influence

beer taste and quality. Yeast cells are usually processed as a thick paste that contains billions of cells which consist of about 75 % water. The composition of the dry matter is as follows: 45 % to 60 % protein, 25 % to 35 % carbohydrate, 4 % to 7 % fats and 6 % to 9 % minerals (phosphates, potassium, sodium, calcium, magnesium, zinc, and traces of iron, manganese and copper) (Kunze, 2004). The breakdown of glucose into ethanol is a result of numerous complex reactions involving several different enzymes that are located in cytoplasm of the yeast cell. Multiplication of these cells occurs by budding, where a small bubble-like protuberance from the mother cell is formed.

The growth of a population of yeasts is well known and defined when pitched to the wort, being divided in six phases (Kunze, 2004). In the first, latent or induction phase, the metabolism of the yeasts is activated, and ends with the first cell division. The second, acceleration phase, is where the rate of cell division continuously increases, reaching the third phase, of exponential growth where the yeast cells are at their most vital. Following that is the deceleration phase in which the growth rate decelerates, possibly due to a reduction in available nutrients or an increase in inhibiting substances as a result of the yeast cell metabolism (Brown *et al.,* 1981). The growth rate continues to decelerate until it reaches a stagnant point, in the stationary phase, where the number of cells formed and cells which die is the same. The last phase is the declining phase, where the rate of cell death exceeds that of cell formation, implying a decrease in amount of yeast cells (Nobile *et al.,* 2003). The duration of each of these phases is primarily influenced by the present substrate, temperature, water content (substrates with at least 15 % water content are required), pH value (preferably acidic pH), oxygen concentration (due to respiration being a bigger energy production source than fermentation, wort is usually aerated in breweries before pitching, in order to improve yeast growth) and physiological condition of the yeast.

Yeasts used in brewing subdivide into many different strains, belonging to two major groups, the top fermenting yeasts (*Saccharomyces cerevisiae*) and the bottom fermenting yeasts (*Saccharomyces pastorianus*) (Capece *et al.*, 2018). The two different types differ on many levels, one such being morphology, as they can be distinguished by budding behaviour with top fermenting yeasts forming chains of budded cells and bottom fermenting occurring as single or pairs of cells. In terms of their metabolism, a very important difference occurs in the fermentation of the trisaccharide raffinose, as bottom fermenting yeast can do it fully, as opposed to top fermenting yeasts, which can use a third of this substance (Tornai-Lehoczki & Dlauchy, 2000). Bottom fermenting yeasts have a lower enzyme content, resulting in a simpler metabolism and flavour of the final product. Their names are given as a result of behaviour shown during fermentation, with bottom fermenting yeasts settling on the bottom toward the end of fermentation, and top fermenting yeasts rising to the top. In regards of fermentation temperature, top fermenting yeasts

proliferate under higher temperatures (14 °C to 25 °C) than bottom fermenting yeasts (4 °C to 12 °C) (Capece *et al.*, 2018). Another noteworthy concept relating to yeast and the brewing world in general is the attenuation yield of a certain yeast. This concept relates to the degree to which yeast ferments the sugar in a wort or must, where having 100 % yield implies a conversion of 100 % of the sugars into ethanol and CO_2 by the yeast.

2.6.1.5. Adjuncts

In addition to the mentioned four raw materials, barley, hops, water and yeast, others can be used in brewing, such as unmalted cereals (maize, rice, barley, sorghum, wheat), sugars such as for example, cane sugar, that is added to the casting wort where it is converted into two monosaccharides that are easily fermentable (Kunze, 2004). Depending on the types of sugar used, colour or flavour can be added to the beer.

2.6.2. Process of production

A part of the beer production process occurs in the brewhouse, which function consists of receiving the main ingredients to produce beer and to process these into a hopped cold wort ready for fermentation (Andrews, 2006). Before entering the brewhouse, barley goes through the process of malting and later milling, with the resulting product going to mashing in the brewhouse. Malting is a process through which barley is modified to green barley. This process involves germination of barley, promoted by water and oxygen, through steeping (immersing barley in water) and aeration, and later, drying (Briggs, 1998). The germination process allows for the development of enzymes in the grain that are capable of converting starch to simple sugars. The crucial enzymes that carry out this conversion are the α-amylase and β-amylase (the latter is already present in the unmalted cereal). The germination of barley promotes secretion of gibberellic acid which initiates production of α-amylase. To avoid overmodification of the grain through these two enzymes, proteases and β-glucanases, the green malt is dried to remove most of the moisture, which stales enzyme activity, formally halting the germination process (Kunze, 2004). In order to stop enzyme activity even further however, another process, curing, is necessary. This process makes use of high temperatures and promotes a reaction between amino acids and sugars that produces melanoidins, conferring flavour and colour to the malt. Several different strategies during these processes are employed to produce special malts like caramel and chocolate, that are used in small proportions in brewing, introducing variations in colour and flavour (Bamforth, 2006).

In the brewhouse, the process initiates with mashing, where malt is mixed with water at high temperatures, and the conversion of starch to fermentable sugars is completed, with other crucial enzymatic activity occurring (Kunze, 2004). Mashing makes use of temperature ramps that promote the activity of specific enzymes according to the intended final product. The enzyme phytase, which diminishes the wort's pH so that other enzymes can work, has a recorded activity from 30 °C to 52 °C, making those the first possible set of temperatures in the mashing process. Next are debranching enzymes, that solubilize starch, and β-glucanase, responsible for the breakdown of β-glucans which increase the difficulty of filtrating the wort (hence optimizing volume of wort produced; this step is usually employed when unmalted cereals are used), both with optimal temperatures from 35 °C to 45 °C. Proteases have a more optimal activity from 45 °C to 55 °C, and these reduce the turbidity of the final product, being responsible for the breakdown of peptones, polypeptides and peptides, and long-chain proteins into medium or short-chain proteins. Following these temperature baselines are the enzymes that convert starch into fermentable sugars, namely maltose and maltotriose, and dextrin (non-fermentable). The enzymes responsible for this breakdown are β-amylase (optimal temperatures of 55 °C to 65 °C, breakdown of starch into maltose), and α-amylase (optimal temperatures of 65 °C to 72 °C, breakdown of starch into mostly maltose, maltotriose and dextrin) (Denault *et al.,* 1981).

In this step wort is produced (sugars are extracted from the malt) and separated from the used grain. The efficiency of this step is augmented by the milling of the malt, promoting a better extraction of sugars with water (Montanari, Floridi *et al.,* 2005). The wort is then separated, going from the mash tun to the lauter tun.

Following separation, the wort is boiled, arresting enzyme activity. The hops are added in this phase, due to the aforementioned isomerization of α-acids that makes possible the bittering of the beer. The wort is usually boiled for 60 min to 90 min, sterilizing it, evaporating undesirable aromas, and precipitating insoluble proteins (O'Rourke, 2002).

The boiled wort is then cooled and fermented, with a conversion of simple sugars to mainly, ethanol and CO_2, producing a green non-maturated beer. This process is carried out by the yeast at a pitch rate designated by the strain used and type of beer intended, usually 0.3 g/L (Kunze, 2004). Several hundreds of organic compounds that characterize beer are formed during fermentation as a result of the complex metabolism of the yeast (White & Zainasheff, 2010), such as esters like isoamyl acetate (banana flavour), ethyl hexanoate (apple), and ethyl acetate, higher alcohols like isoamyl alcohol (3-methyl-1-butanol) and 2-phenylethanol, acids like octanoic, butyric, isovaleric and acetic. Other compounds are dialkyl sulphides such as dimethyl sulphide, and diketones such as diacetyl. Some off-flavours are a result of the yeast

metabolism, for example *trans*-2-nonenal – stale and oxidized flavours (Noël *et al.*, 1999) – but can be worked around (albeit with difficulty, as the said metabolism is still not completely understood) through adapting to the conditions that promote the formation of these compounds (Pires & Brányik, 2015). The temperatures at which fermentation occurs are defined by the brewer, according to the style of beer intended and strain of yeast used.

After fermentation, processes such as maturation (which is vital to eliminate diacetyl), secondary fermentations, carbonation and finally packaging are followed. Maturation comprehends all the transformations that occur between the end of primary fermentation and final filtration of the beer, these being carbonation by fermentation of residual sugars, removal of excess yeast by sedimentation, adsorption on the surface of the yeast of various non-volatile materials, reduction of the haze-forming potential by precipitation of protein/polyphenol complexes and progressive changes in flavour and aroma (Masschelein, 1986; Alves, 2020). This process varies in time and temperatures required with the desired effect and type of beer, but usually a range of 7 to 30 days applies (Brányik, 2005), with temperatures from 0 °C to 10 °C, where colder temperatures influence beer clarity positively and higher temperatures shorten the process (Aroh, 2019). Carbonation is a very important process that affects the quality of the final product, where levels of CO_2 that do not match a particular style of beer may decrease its organoleptic qualities. This process is generally implemented through forced carbonation where a pressure of CO_2 is applied to match the temperature of the product, according to a table of predefined values, as shown in Figure 3.

2.7. Food Safety – Quality Control

Legislative and commercial requirements gave rise for the need to implement systems that assure the safety, quality and legality of beer production (Bamforth, 2006). These systems are the hazard analysis and critical control points (HACCP), and the quality management systems, ISO 9001. The HACCP system has become a method used in breweries worldwide and is based on an organized, systematic, scientific, preventive assessment, identifying hazards and the probability of their occurrence throughout all stages of the production process, ensuring and controlling the safety of foodstuffs and consequently consumers throughout the entire food chain (Novais, 2006). The HACCP methodology constitutes an accepted international reference for the implementation of Food Safety Systems, allowing companies to easily comply with legal requirements, promoting the trust and safety of their products and being prepared for certification (Worsfold, 2001).

p/bar; *p*/(100 kPa)

T/°C

	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20	1.25	1.30	1.35	1.40	1.45	1.50	1.55	1.60	1.65	1.70	1.75
-0.5	2.10	2.18	2.26	2.34	2.43	2.51	2.59	2.67	2.75	2.83	2.91	2.99	3.07	3.15	3.23	3.31	3.39	3.47	3.55	3.63	3.71	3.79	3.87	3.95	4.03	4.11	4.19	4.27	4.35	4.43
0.0	2.06	2.14	2.22	2.30	2.38	2.45	2.53	2.61	2.69	2.77	2.85	2.93	3.00	3.08	3.16	3.24	3.32	3.40	3.47	3.55	3.63	3.71	3.79	3.87	3.95	4.02	4.10	4.18	4.26	4.34
0.5	2.02	2.10	2.17	2.25	2.33	2.41	2.48	2.56	2.64	2.71	2.79	2.87	2.94	3.02	3.10	3.18	3.25	3.33	3.41	3.48	3.56	3.64	3.71	3.79	3.87	3.94	4.02	4.10	4.18	4.25
1.0	1.98	2.06	2.13	2.21	2.28	2.36	2.43	2.51	2.59	2.66	2.74	2.81	2.89	2.96	3.04	3.11	3.19	3.26	3.34	3.42	3.49	3.57	3.64	3.72	3.79	3.87	3.94	4.02	4.09	4.17
1.5	1.94	2.02	2.09	2.17	2.24	2.31	2.39	2.46	2.54	2.61	2.68	2.76	2.83	2.91	2.98	3.05	3.13	3.20	3.28	3.35	3.42	3.50	3.57	3.65	3.72	3.79	3.87	3.94	4.02	4.09
2.0	1.91	1.98	2.05	2.13	2.20	2.27	2.34	2.42	2.49	2.56	2.63	2.71	2.78	2.85	2.92	3.00	3.07	3.14	3.21	3.29	3.36	3.43	3.50	3.58	3.65	3.72	3.80	3.87	3.94	4.01
2.5	1.87	1.94	2.01	2.09	2.16	2.23	2.30	2.37	2.44	2.51	2.58	2.66	2.73	2.80	2.87	2.94	3.01	3.08	3.16	3.23	3.30	3.37	3.44	3.51	3.58	3.65	3.73	3.80	3.87	3.94
3.0	1.84	1.91	1.98	2.05	2.12	2.19	2.26	2.33	2.40	2.47	2.54	2.61	2.68	2.75	2.82	2.89	2.96	3.03	3.10	3.17	3.24	3.31	3.38	3.45	3.52	3.59	3.66	3.73	3.80	3.87
3.5	1.81	1.87	1.94	2.01	2.08	2.15	2.22	2.29	2.36	2.42	2.49	2.56	2.63	2.70	2.77	2.84	2.91	2.97	3.04	3.11	3.18	3.25	3.32	3.39	3.46	3.52	3.59	3.66	3.73	3.80
4.0	1.77	1.84	1.91	1.98	2.04	2.11	2.18	2.25	2.31	2.38	2.45	2.52	2.58	2.65	2.72	2.79	2.85	2.92	2.99	3.06	3.13	3.19	3.26	3.33	3.40	3.46	3.53	3.60	3.67	3.73
4.5	1.74	1.81	1.88	1.94	2.01	2.08	2.14	2.21	2.27	2.34	2.41	2.47	2.54	2.61	2.67	2.74	2.81	2.87	2.94	3.01	3.07	3.14	3.20	3.27	3.34	3.40	3.47	3.54	3.60	3.67
5.0	1.71	1.78	1.84	1.91	1.98	2.04	2.11	2.17	2.24	2.30	2.37	2.43	2.50	2.56	2.63	2.69	2.76	2.82	2.89	2.95	3.02	3.09	3.15	3.22	3.28	3.35	3.41	3.48	3.54	3.61
5.5	1.69	1.75	1.81	1.88	1.94	2.01	2.07	2.14	2.20	2.26	2.33	2.39	2.46	2.52	2.58	2.65	2.71	2.78	2.84	2.91	2.97	3.03	3.10	3.16	3.23	3.29	3.35	3.42	3.48	3.55
6.0	1.66	1.72	1.78	1.85	1.91	1.97	2.04	2.10	2.16	2.23	2.29	2.35	2.42	2.48	2.54	2.61	2.67	2.73	2.80	2.86	2.92	2.98	3.05	3.11	3.17	3.24	3.30	3.36	3.43	3.49
6.5	1.63	1.69	1.76	1.82	1.88	1.94	2.00	2.07	2.13	2.19	2.25	2.32	2.38	2.44	2.50	2.56	2.63	2.69	2.75	2.81	2.87	2.94	3.00	3.06	3.12	3.19	3.25	3.31	3.37	3.43
7.0	1.61	1.67	1.73	1.79	1.85	1.91	1.97	2.03	2.10	2.16	2.22	2.28	2.34	2.40	2.46	2.52	2.58	2.65	2.71	2.77	2.83	2.89	2.95	3.01	3.07	3.13	3.20	3.26	3.32	3.38
7.5	1.58	1.64	1.70	1.76	1.82	1.88	1.94	2.00	2.06	2.12	2.18	2.24	2.30	2.36	2.42	2.48	2.54	2.60	2.66	2.72	2.79	2.85	2.91	2.97	3.03	3.09	3.15	3.21	3.27	3.33
8.0	1.56	1.62	1.68	1.73	1.79	1.85	1.91	1.97	2.03	2.09	2.15	2.21	2.27	2.33	2.39	2.45	2.51	2.56	2.62	2.68	2.74	2.80	2.86	2.92	2.98	3.04	3.10	3.16	3.22	3.28
8.5	1.53	1.59	1.65	1.71	1.77	1.83	1.88	1.94	2.00	2.06	2.12	2.18	2.23	2.29	2.35	2.41	2.47	2.53	2.58	2.64	2.70	2.76	2.82	2.88	2.93	2.99	3.05	3.11	3.17	3.23
9.0	1.51	1.57	1.63	1.68	1.74	1.80	1.86	1.91	1.97	2.03	2.09	2.14	2.20	2.26	2.32	2.37	2.43	2.49	2.55	2.60	2.66	2.72	2.78	2.83	2.89	2.95	3.01	3.06	3.12	3.18
9.5	1.49	1.55	1.60	1.66	1.72	1.77	1.83	1.89	1.94	2.00	2.06	2.11	2.17	2.23	2.28	2.34	2.40	2.45	2.51	2.57	2.62	2.68	2.74	2.79	2.85	2.91	2.96	3.02	3.08	3.13
10.0	1.47	1.52	1.58	1.63	1.69	1.75	1.80	1.86	1.91	1.97	2.03	2.08	2.14	2.19	2.25	2.30	2.36	2.42	2.47	2.53	2.58	2.64	2.70	2.75	2.81	2.86	2.92	2.98	3.03	3.09
10.5	1.45	1.50	1.56	1.61	1.67	1.72	1.78	1.83	1.89	1.94	2.00	2.05	2.11	2.16	2.22	2.27	2.33	2.38	2.44	2.49	2.55	2.60	2.66	2.71	2.77	2.82	2.88	2.93	2.99	3.04
11.0	1.43	1.48	1.53	1.59	1.64	1.70	1.75	1.81	1.86	1.91	1.97	2.02	2.08	2.13	2.19	2.24	2.29	2.35	2.40	2.46	2.51	2.57	2.62	2.67	2.73	2.78	2.84	2.89	2.95	3.00
11.5	1.41	1.46	1.51	1.57	1.62	1.67	1.73	1.78	1.83	1.89	1.94	2.00	2.05	2.10	2.16	2.21	2.26	2.32	2.37	2.42	2.48	2.53	2.58	2.64	2.69	2.74	2.80	2.85	2.91	2.96
12.0	1.39	1.44	1.49	1.55	1.60	1.65	1.70	1.76	1.81	1.86	1.92	1.97	2.02	2.07	2.13	2.18	2.23	2.28	2.34	2.39	2.44	2.50	2.55	2.60	2.65	2.71	2.76	2.81	2.87	2.92
12.5	1.37	1.42	1.47	1.52	1.58	1.63	1.68	1.73	1.79	1.84	1.89	1.94	1.99	2.05	2.10	2.15	2.20	2.25	2.31	2.36	2.41	2.46	2.51	2.57	2.62	2.67	2.72	2.78	2.83	2.88
13.0	1.35	1.40	1.45	1.50	1.56	1.61	1.66	1.71	1.76	1.81	1.86	1.92	1.97	2.02	2.07	2.12	2.17	2.22	2.28	2.33	2.38	2.43	2.48	2.53	2.58	2.64	2.69	2.74	2.79	2.84
13.5	1.33	1.38	1.43	1.48	1.54	1.59	1.64	1.69	1.74	1.79	1.84	1.89	1.94	1.99	2.04	2.09	2.14	2.20	2.25	2.30	2.35	2.40	2.45	2.50	2.55	2.60	2.65	2.70	2.75	2.80
14.0	1.32	1.37	1.42	1.47	1.52	1.57	1.62	1.67	1.72	1.77	1.82	1.87	1.92	1.97	2.02	2.07	2.12	2.17	2.22	2.27	2.32	2.37	2.42	2.47	2.52	2.57	2.62	2.67	2.72	2.77
14.5	1.30	1.35	1.40	1.45	1.50	1.55	1.60	1.64	1.69	1.74	1.79	1.84	1.89	1.94	1.99	2.04	2.09	2.14	2.19	2.24	2.29	2.34	2.39	2.44	2.49	2.53	2.58	2.63	2.68	2.73
15.0	1.28	1.33	1.38	1.43	1.48	1.53	1.58	1.62	1.67	1.72	1.77	1.82	1.87	1.92	1.97	2.01	2.06	2.11	2.16	2.21	2.26	2.31	2.36	2.40	2.45	2.50	2.55	2.60	2.65	2.70
15.5	1.27	1.31	1.36	1.41	1.46	1.51	1.56	1.60	1.65	1.70	1.75	1.80	1.84	1.89	1.94	1.99	2.04	2.09	2.13	2.18	2.23	2.28	2.33	2.38	2.42	2.47	2.52	2.57	2.62	2.66
16.0	1.25	1.30	1.35	1.39	1.44	1.49	1.54	1.58	1.63	1.68	1.73	1.77	1.82	1.87	1.92	1.96	2.01	2.06	2.11	2.16	2.20	2.25	2.30	2.35	2.39	2.44	2.49	2.54	2.58	2.63
16.5	1.24	1.28	1.33	1.38	1.42	1.47	1.52	1.56	1.61	1.66	1.71	1.75	1.80	1.85	1.89	1.94	1.99	2.04	2.08	2.13	2.18	2.22	2.27	2.32	2.36	2.41	2.46	2.51	2.55	2.60
17.0	1.22	1.27	1.31	1.36	1.41	1.45	1.50	1.55	1.59	1.64	1.69	1.73	1.78	1.82	1.87	1.92	1.96	2.01	2.06	2.10	2.15	2.20	2.24	2.29	2.34	2.38	2.43	2.48	2.52	2.57

Figure 3. Forced carbonation chart for beer, relating temperature (*T*) with the pressure (*p*) (adapted from O'Leary, 2008). Blue-under carbonated; Gray-lightly carbonated (British ales, stouts and porters); Green-moderately carbonated (lagers, ales, most beers); Yellow-highly carbonated (lambics and wheat beers); red-over carbonated (certain types of ales).

The HACCP relates the quality of raw materials, processes, product composition, storage conditions, type of packaging, among others, based on seven principles (Baptista, Pinheiro, & Alves, 2003; Mortimore & Wallace, 2013), according to the Codex Alimentarius:

1. Carry out a risk analysis;
2. Determine critical control points (CCPs);
3. Establish critical threshold(s);
4. Establish a system to monitor the control of CCPs;
5. Establish corrective action to be taken when monitoring indicates that a specific CCP is not under control;
6. Establish verification procedures to confirm that the HACCP system works effectively;
7. Establish documentation regarding all procedures and records appropriate to these principles and their application.

In Portugal, it was implemented with the decree 67/89 of March 18th, which establishes the general rules for the hygiene of foodstuffs, as well as the modalities for verifying compliance with these rules, which was later amended by decree 425/99 of October 21st, 1999. This decree was revoked in decree 113/2006, however, the enforcement of HACCP practices is present in EC Regulation no. 852/2004.

The ISO 9001 on another hand is a quality management system and deals only with the quality of a product, defining activities for which the brewery must provide appropriate control, but not how these aspects must be controlled (Tarí, Molina-Azorín, & Heras, 2012). This Standard is also present in most breweries nowadays, complementing the activity of other systems in place such as the HACCP to offer quality and security to the consumer.

Quality examinations of beer are usually in order, as to assure consumers of its quality and safety, and can be done by beer tasting, microbiological monitoring, and chemical and physical properties examination. Through beer tasting, many substances can be perceived, as well as properties of the finished product such as the amount and stability of the foam, colour, and turbidity (Parker, 2012). Quality determining parameters such as taste and aroma however are more difficult to measure, and in order to draw specific conclusions, a specific and strict method must be put in place. These analyses fall under the category of sensory evaluation, and include dilution tests, sweetness comparison tests, bitterness comparison tests, duo-trio tests and triangular tests. Usually, the sensory evaluation performed in breweries is done among the available workforce, which with experience make increasingly more correct distinctions and conclusions. Other tests are also applied, among the consumer as to get their perspective on the product, namely, preference and acceptance tests (Costell, 2002). In the case of consumers, descriptive tests could also be applied, at the risk of losing reliability of results, as hundreds of descriptors can be used for beer.

The microbiological examination is another form of quality control, as it is possible for microorganisms that cause spoilage to pass from the production line to the finished product (Kunze, 2004). If not detected in early stages these can make beer turbid and produce substances through their metabolic pathways that spoil its taste. In regard to microorganisms that could be found in beer, three categories exist, the indirect beer spoilage organisms (harmless accompanying microorganisms), the potentially damaging microorganisms, and the obligatory spoilage microorganisms. The first usually cannot grow in beer and consequently die, serving however as indicators to the presence of undesirable microorganisms. The potentially damaging (*Lactobacillus casei*, *Lactococcus lactis* and Enterobacteriacea) are the microorganisms that can grow on beer given that certain conditions are met, such as oxygen content, low pH and bitterness, which makes these attributes important factors to control (Schneiderbanger, Grammer, Jacob, & Hutzler, 2018). The obligatory microorganisms in beer (*Pectinatus cerevisiiphilus* and *Megasphaera cerevisiae*) are singled out due to their particularly damaging effect and favourable conditions for growth in beer (Kunze, 2004). Frequent checks are preferable in breweries at several segments of the production line as a means to determine where and how microorganisms enter the

brewing line, and to eliminate them accordingly, with countermeasures being introduced. However, at smaller breweries, beer quality can be difficult to ensure through microbiological monitorization, and so, physical and chemical properties examinations attain a higher prevalence. These examinations focus on a variety of parameters and are done along several steps of beer production (Bamforth, 2006). They include analysis of exctract concentration, determination of pH, diacetyl content, bitterness units, CO_2 content, colloidal stability and other measurements. Determination of extract concentration and pH along processes such as mashing, boiling and fermentation also help keep these values in check for the specific style of beer being brewed (Habschied, Mastanjević, 2021).

2.8. Beer types

Beer can be classified according to many factors, the main being type of fermentation, with top fermenting beers and bottom fermenting beers (Capece *et al.*, 2018). Top fermenting beer yeasts develop larger amounts of fermentation by-products, therefore conferring a more complex aromatic profile to the beer. The styles of beer included in this category are wheat beers, Berliner Weisse, Altbier, Kölsch, Ale, Stout, Porter, Trappist and Lambic (Bamforth, 2006). On the other hand, bottom fermenting yeasts confer a cleaner profile to the fermented product, and styles of beer included in this category are the Pilsner, Lager, Bockbier and Doppelbock beers (Bamforth, 2006).

2.8.1. Grape Ales and Lagers

Ale beers are produced usually with well modified malts and adjuncts, which in conjunction with the complex aromatic profile conferred by the yeast give a more complex final product. The alcohol content, by volume (*ABV* – alcohol by volume), of these beers can range from 3 % to 10 % and the bitterness varies greatly, with Indian Pale Ale being very heavily hopped, and mild ales being sweeter (Bamforth, 2006). Lager beers account for 80 % to 90 % of bottom fermenting beers and are characterized by an *ABV* between 4.7 % and 5.3 % and a moderate bitterness (*IBU* from 18 to 23) (Bamforth, 2006).

The Grape Ale and Lager are therefore derivatives of these styles that involve an addition of grapes or grape must. The most notorious beer style that involves the addition of grape must is the IGA, Italian grape ale (Garavaglia, 2018), defined as a communion between beer and wine (Garavaglia, 2020), whose pioneer, Nicola Perra, used *sapa*, grape must syrup obtained by long boiling. Since then, the main raw materials were conventionally defined as pils or pale malt, with possibly some adjuncts or special malts. The grape must used (pasteurized or not) can be introduced at different stages and different quantities with the final product varying highly. This addition can occur at boil, primary/secondary fermentation, or

aging, with quantities ranging from 5 % to 10 % of the final volume, although, they can go up to quantities such as 40 %, all depending on the creative decision of the author of the beer, since despite existing a norm for this style, no set of rules is actually defined, making this style highly experimental and ever-evolving (Marin *et al.*, 2021). Also, a factor of this variety in the style is the yeast used and therefore fermentation profile, which can be neutral or fruity. A wide range of hops can also be used, albeit at low amounts as not to give the final product an excessive beer character. Another interesting aspect of these beer styles is their interconnection with sour beers, as often grape ales could be soured, due to the already present acidic element of the grape must.

As for standard quality parameters, the original extract (*°Plato*) is usually between 10.7 and 22.5 with a final extract of 1.7 to 3.7, bitterness (*IBU*) between 10 and 30, colour (*EBC*) between 10 and 60, and *ABV* ranging from 4.8 % to 10 % (de Francesco, Marconi *et al.* 2021). The wide range of the different parameters indicates the complexity of this style (Alfeo, Todaro *et al.*, 2019). This is a style complicated to define as various materials and various characteristics interplay, being therefore a style very subject to innovation and change, where multiple of the above-defined parameters can be changed for a significantly different final product (de Francesco, Marconi *et al.* 2021).

Given the acidic character that the grape must confers, beers produced with this component are sometimes paired with souring techniques in order to produce complex sour beers. The sour beer is known as a beer with intentionally sour taste where, traditionally, the acidic character is the result of spontaneous, mixed fermentation, implying a variety of bacteria and yeast species participation. A very popular style that is a result of this more traditional souring is the lambic beer. The traditional fermentation used to produce them does not apply pure yeast cultures but relies on spontaneous, environmental inoculation. The fermentation and maturation process of water, barley malt, unmalted wheat, and aged dry hops is carried out in horizontal oak or chestnut barrels (de Roos & de Vuyst, 2022) and can take up to three years, resulting in a sour, fruity beer with a complex microbial consortium and flavour profile that not only can be drunk as such but also serves as the basic product for other, lambic-derived beers (Bongaerts *et al.*, 2021). It is characterized by different microbial species belonging to the enterobacteria, acetic acid bacteria, lactic acid bacteria, and yeasts (de Roos & de Vuyst, 2019). However, the difficulty in maintaining a standardized quality due to the spontaneous aspect of the fermentation, paired with the time it takes to finish production gave rise to a different souring technique, an addition of pure acidifying organisms chosen with the desired characteristics in mind. These organisms, such as non-*Saccharomyces* yeasts (*e.g. Brettanomyces*) or acidifying bacteria (*e.g.* lactic acid bacteria, acetic acid bacteria) can be used before (pre-alcoholic fermentation) or after (secondary fermentation) the main

fermentation with brewer's yeast, resulting in differing final products. Acidification through pre-alcoholic fermentation with lactic acid bacteria is a known method used nowadays, which contributes to the sensory profile of the beer not only through the production of lactic acid, but also regarding volatile compounds and organic acids (Dysvik *et al.*, 2019).

3. Materials and Methods

With the goal of producing a grape-based beer three main product tests were conducted which led to a final test solely made from the conclusions drawn from the previous three. The three main product tests served as a basis to mainly test three different styles of beer through which a final one was selected. Also, several other factors were tested, here considered secondary aspects, such as malts, hops, and techniques used (*e.g.* kettle souring and dry hopping). These secondary aspects were not kept isolated from test to test like the primary aspect, the style of beer, meaning that knowledge gained on the first product test, the Grape Ale, was used to better develop these aspects on the second product test, the Grape Lager, and then again for the third, the Grape Weiss.

Finalized these three main tests, both the secondary aspects refined throughout the product tests, and the primary aspect, the beer style, were imparted to the final product test, as seen in Figure 4. Here the work carried out in this study is summarized, the dashed lines implying the improvement of the secondary aspects along the course of the study, and the arrows meaning a possibility for a chosen beer style, which was decided after production of the Grape Ale, Grape Lager and Grape Weiss.

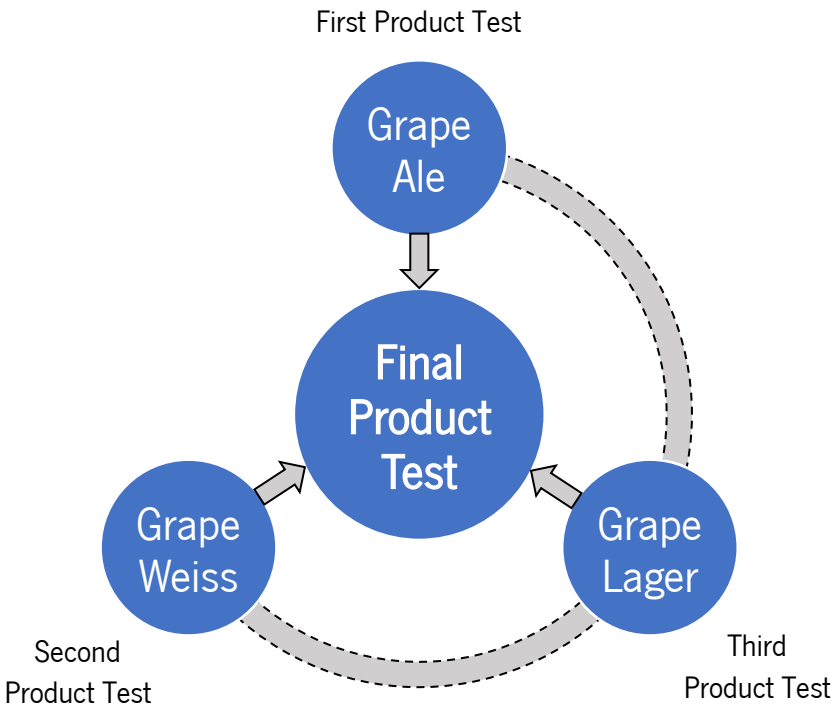

FIGURE 4. ORGANIZATION OF THE STUDY.

3.1. Brewing and fermentation equipment

An equipment that simulates the process of the brewhouse was used for brewing, the Grainfather, which is an all-in-one brewing system that allows the use of crushed grain, and mash, sparge and cool within the same unit. It is therefore an equipment fit for experimenting and innovating. Built into the boiler is a temperature control, heating elements, recirculating pump and counter flow chiller.

The Grainfather is made of materials that grant the quality required for the brewing process such as 304 grade stainless steel, tempered glass, magnetic drive pump, copper cooling coil, etc. It has a 30 L capacity, meaning it can produce approximately 23 L of craft beer at one time, being also dotted with a magnetic drive pump operating at a rotational speed of 1800 min^{-1}, that helps extract all sugars in the grain giving optimal brewing efficiency.

After boiling, the wort is cooled through the use of the aforementioned counter flow chiller directly onto a separate fermenter. Here, temperature can be measured, along with visual verification of fermentation. Additionally, samples were taken through the use of a hose, allowing for sensory evaluation (tasting) and measurements of pH and extract concentration, as *°Plato* (where a *°Plato* of 10 corresponds to an extract concentration of 100 g/kg) during the production and fermentation process. Evaluation of pH and extract concentrations were also made during fermentation, and for both brewing and fermentation, samples were collected, cooled for a time to room temperature and analysed with pH-meter Model 20 (Crison) for pH and Refractometer PAL-1 (Atago) for extract concentration where values are obtained in *Brix*, which were later converted to *°Plato*. Measures of CO_2, O_2 and *ABV* (alcohol by volume) were also made of the finished product, with a CboxQC AT-line and filling device PFD (Anton Paar) for CO_2 and O_2, and an alcohol analysing system, Alcolyzer (Anton Paar), for *ABV*.

3.2. Raw Materials

3.2.1. Malts

Three different types of malted products were used depending on the beer type, Finest Lager (from Simpsons Malt), Cara Aroma (from Weyerman) and Wheat Malt (from Simpsons Malt). Technical specifications for malted and unmalted cereals used are present in Appendix A, Table A1, and amounts used in the different product tests are in Table 3.

3.2.2. Hops

In terms of hops, Citra hops (from Yakima Chief Hops) and Tettnang Tettnanger (from Charles Faram) were used. Technical specifications for hops used are present in Appendix A, Table A2. Table 3 presents the amounts used for the different product tests.

3.2.3. Yeasts and Bacteria

Yeasts were again used accordingly with the different beer types (Table 3), and the main focus for this study were the SafAle™ US-05 (Fermentis), SafLager™ W-34/70 (Fermentis), SafAle™ T-58 (Fermentis). Other than these, an acidifying bacterium was used, Wildbrew™ Helveticus pitch (Lallemand), and a yeast typically found in wine production, Lalvin QA23™ (Lallemand).

3.2.4. Adjuncts

Among the adjuncts vital for these product tests (Table 3) were the grape must from the grape noble variety of Loureiro courtesy of Esporão's Quinta do Ameal, Flaked Oats (from Simpsons Malt), Flaked Wheat (from Simpsons Malt), Go-Ferm Protect™ (Lallemand), DAP, or diammonium phosphate, Servomyces (Lallemand), and $CaCO_3$ and Citric acid (Sameca).

3.2.5. Salts

Salts used included only $CaCl_2$ and $CaSO_4$ (Sameca) with amounts specified in Table 3.

3.3. Production Process

The four product tests followed the same procedure with small differences. For beer production with the Grainfather equipment the malt was weighed and milled, in anticipation to the mashing, and added to the brewing equipment alongside water from a reverse osmosis system (volume of water is specified in Table 3). Mashing followed this first step, with different temperature ranges for the different product tests according to the mashing guidelines in Table 3, and salts were added. The final product test followed the same mashing guidelines as the Grape Lager. Grape must was added in this step for the Grape Ale only, in this case half of the total amount of must used, 5 L. With the brewing equipment used, recirculation of the wort occurred during the whole process of mashing, at the end of which the wort was filtered. After filtration, boiling procedures take place, at 100 °C for 60 min for the first three products, and 90 min for the final product test. With the grape ale only, a first boiling occurred before kettle souring, for 15 min, with an addition of lactic acid bacterium (*Lactobacillus helveticus)* after the wort cooled to 43 °C. The

souring step ended when the wort hit the target pH of 3.39. Grape must was mainly added in this step, only 5 L for the Grape ale, and 10 L for the rest, all at 15 min remaining in the boiling and represented 43.5 % of the mashing volume. Hops were also added in this step, with 20 min remaining for the Grape Ale, and 60 min for the other three product tests. The sterilized wort then proceeded to cooling with the counter flow chiller and sent into a fermentation vessel, where the yeast was added in a uniform manner through direct pitching. For the Grape Weiss and final product test, an additional use of yeast was performed to restart fermentation. The contents were moved to refrigeration at the end of fermentation and dry hop additions were made for the Grape Ale and final product test (its second dry hop addition). For this final product test, a cleaning of H_2S aroma was necessary and carried out before refrigeration by passing the maturing beer through a copper surface (as well as a first dry hop addition). Maturation occurred at 7 °C for 15 days for the Grape Ale and Grape Weiss, 20 days for the Grape lager and 30 days for the final product test. Finally, after priming with CO_2, bottling procedures took place. During fermentation and maturation, samples were taken when possible for the measurement of temperature, *°Plato* and pH. Specifications for the amounts of raw materials used in each product test are represented in Table 3. The represented dosages of yeasts and adjuncts (percentage of grape must, DAP, Servomyces and Go-ferm) are the planned concentrations for each product test and not the exact values as data on the final volume of the wort both in mashing and boiling is unavailable.

3.4. Sensory Analysis

Sensory analysis trials used in order to better perceive the consumer's point of view on the products fabricated was an acceptance test, where a grading of 1-5 on a hedonic scale was requested of 15 panellists, 11 men and 4 women, from the ages of 22 to 58 on the categories of appearance, aroma, bitterness, flavour, palatefulness and global appreciation, and an answer of "Yes/No" on the category of intent to purchase according to the evaluation sheet designed, adapted from an existing Sovina's evaluation sheet (Figure 5). The panellists ranged from people with experience on the beer industry and therefore more trained to understand subtle differences in the product, to others who were not trained. Also, an important factor in these sensory analysis assays were the varied preferences in the panellists and their like/dislike of beer as a product in general. They were implemented 2 h before lunch and crackers and water were served so that the palate could be cleansed in between the different beers. For the first sensory evaluation, the three first product tests were analysed, and the beers were served according to the same order to all panellists, going as follows: Grape Ale, Grape Lager and lastly, Grape Weiss. Characteristics for these products were not given on an initial phase to recreate a blind test and

panellists only had to grade the appearance, aroma, bitterness and palatefulness, filling the flavour category with a descriptive term of their choice.

Table 3. Raw materials and process guidelines used for the product tests

		Grape Ale		Grape Lager		Grape Weiss		Final Product Test	
Salts		$CaCl_2$	2 g	$CaCl_2$	2 g	$CaCl_2$	2 g	$CaCl_2$	2 g
		$CaSO_4$	1 g	$CaSO_4$	1 g	$CaSO_4$	1 g	$CaSO_4$	1 g
Malts		Finest Lager	2.0 kg	Finest Lager	3.5 kg	Finest Lager	2.0 kg	Finest Lager	7.0 kg
		Cara aroma	0.2 kg	Flaked Oats	0.7 kg	Wheat Malt	2.0 kg	Wheat Flakes	1.0 kg
Hops		Citra	20 g	Citra	13 g	Tettnanger	20 g	Citra	15 g
		Citra (dry hopping)	10 g					Citra (dry hopping)	10 g
								Citra (dry hopping)	10 g
Yeast/Bacteria		US-05	11.5 g (52.2 g/hL)	W-34/70	11.5 g (52.2 g/hL)	T-58	23.0 g (104 g/hL)	W-34/70	23.0 g (104 g/hL)
		Helveticus pitch	2.00 g (10.0 g/hL)					QA23	11.0 g (50.0 g/hL)
Adjuncts		Grape Must	5 L (in mashing)	Grape Must	10 L	Grape Must	10 L	Grape Must	10 L
			5 L (in boiling)						
		GoFerm	2.85 g (12.9 g/hL)	GoFerm	3.60 g (16.4 g/hL)	Servomyces	1.00 g (4.54 g/hL)	Servomyces	1.00 g (4.54 g/hL)
		DAP	2.85 g (12.9 g/hL)	DAP	3.00 g (13.6 g/hL)	DAP	3.00 g (13.6 g/hL)	DAP	3.00 g (13.6 g/hL)
Water	Mashing	12.0 L		20.0 L		8.0 L		28.0 L	
	Sparging	3.0 L		5.0 L		6.5 L		–	
CO_2		2.6 g/L		2.6 g/L		2.6 g/L		2.6 g/L	
Mashing guidelines		64 °C	60 min	64 °C	30 min	55 °C	15 min	64 °C	30 min
		78 °C	15 min	68 °C	30 min	64 °C	45 min	68 °C	30 min
				75 °C	10 min	76 °C	15 min	75 °C	10 min
Boiling Guidelines		100 °C	60 min	100 °C	60 min	100 °C	60 min	100 °C	90 min

On a second phase, the characteristics of the beer were given to the panellists, and a completion of the grading (flavour, global appreciation and intent of purchase) was achieved. The second sensory evaluation trial was conducted at a later date and only the final product test was evaluated.

Sensory Analysis Evaluation Sheet

Name	Date

On a first stage, evaluate the appearance, aroma, bitterness and palatefulness categories with a 1-5 scale based on personal preference. For the flavour category utilize only a descriptor and await further instructions.

Score (1-5)			
Product	A	B	C
Appearance			
Aroma			
Bitterness			
Flavour			
Palatefulness			

After a description of the product is given evaluate with a 1-5 scale based on preference the flavour category taking into account the characteristics of the product.

Final Scoring			

Intent to Buy (Yes/No)			

Tasting Notes:

FIGURE 5. Acceptance test evaluation sheet (Adapted from Sovina's sensory evaluation sheet).

3.5. Statistical Analysis

In order to assess the statistical differences between the four product tests the Friedman test (a non-parametric test, meaning it is not conditioned by any probability distribution of the data under analysis, as is expected in the case of the acceptance variable) was used and calculations were made through

Excel at the significance level of α=0,05. With this test, samples were considered different if the test statistic (Eq. 1) was superior to that of tabulated values in Meilgaard, Carr *et al.* (2016).

$$T = \left(\frac{12}{b \times t \times (t+1)} \sum x^2\right) - 3 \times b \times (t+1) \qquad \text{(Eq. 1)}$$

Following the Friedman test, the samples were compared two by two using the Fisher's Least Significant Difference (*LSD*) test. In this, two samples were deemed different if the difference between their sums was greater than the LSD_{rank} calculated in Excel (Eq. 2).

$$LSD_{\text{rank}} = z_{\alpha/2}\sqrt{bt(t+1)/6} = t_{\alpha/2,\infty}\sqrt{bt(t+1)/6} \qquad \text{(Eq. 2)}$$

3.6. Industrial Plan

The industrial plan was set according to the production guidelines present at Sovina's factory, and the HACCP plan drawn was adapted from the company's own HACCP plan.

4. Results and Discussion

The results and discussion were organized into study on the production of grape-based craft beer, subchapter 4.1, where the results of the Grape Ale, Grape Lager and Grape Weiss beers are presented and discussed in three steps. Firstly, the production process is justified and discussed, then, on a second phase, a description of the final product is given (assessed by the author of the study at the time of production), and finally, the results of the sensory evaluation trials for the specific product is given (acceptance test results on the five characteristics evaluated, global appreciation and intent of purchase). Following this, in subchapter 4.2, the same structure of results discussion is used, for the last product test, which acts as the culmination of the study since all the previous product tests contributed to it. Following this main focus of the study comes the future tests, subchapter 4.3, and industrial plan, subchapter 4.4, which covers the adaptation of the production process described in the materials and methods section to the industrial scale, as well as an HACCP plan, where Sovina's factory and HACCP plan both served as models.

4.1. Study on the Production of Grape-based Craft Beer

4.1.1. First Product Test – Grape Ale

With the Grape Ale product test, the goal was to obtain a final product as complex as possible, in order to decide which elements do not work with a grape-based beer product, using a variety of techniques such as the kettle sour and the high krausen dry hopping (late addition of hops during the most active point of fermentation). For the fermentation type tested with this product, the high fermentation of an ale was chosen, which implies a higher complexity in malts used and yeast metabolism. Besides the basic malt, finest lager, a special malt was used, cara aroma, responsible for a caramel taste and a darker colour in the final product, and grape must, in amounts corresponding to 43.5 % of the planned mashing volume.

Finest Lager is a low colour and well modified lager malt. It is a high extract UK spring two row barley with a high enzymic activity, low colour and low nitrogen (Simpsons Malt, 2022a). Cara aroma on the other hand is a brown, richly aromatic and caramelized malt, usually used for ales on account of enzymatic modification, that gives the final product notes of roasted nuts, dark caramel, and dried fruit (Weyermann, 2022). The grape must utilized was of the white grape noble variety of Loureiro courtesy of Esporão's Quinta do Ameal, situated in the region of 'Vinhos Verdes', and known for the growing of this particular noble variety. The aroma from Loureiro grapes is based on quantities of linalool above its perception threshold, which makes this cultivar an aromatic variety (Oliveira, 2001).

Mashing occurred for 60 min at 64 °C with a mash out at 78 °C, and salts such as $CaCl_2$ and $CaSO_4$ were added to correct mineral deficiencies attained through the use of de-mineralized water, to the water being heated for mashing, and are essential for the yeast metabolism. These salts provide calcium ions (the use of both helps balance the sulphate and chloride anions) essential for enzyme function, kettle protein coagulation and yeast metabolism.

The kettle sour used is a process meant to sour the final product and it differs from a traditional mash in the sense that beer is boiled, cooled, and dosed with lactic acid bacteria, in this case, a homofermentative strain of *Lactobacillus helveticus* which is a high-performance, high-purity lactic acid bacteria ideal for a wide range of sour beer styles, producing an intense and sharp citrus flavour profile. Optimal temperature range is 38 °C to 45 °C, and under these conditions this bacterium causes a fast pH drop (in this case 4.59 to 3.39 in three days), and a high production of lactic acid and low acetic acid (Lallemand Brewing, 2022a). *Lactobacillus helveticus* was inoculated on the unhoped wort (hop presence inhibits growth of the bacteria) through the kettle souring technique at a dosage of 10 g/hL as indicated by the manufacturer.

After the souring process hit a target pH of 3.39 the liquid proceeded to boiling, and hops were added. Citra hops feature a high α-acid and total oil count (with a relatively low percentage of cohumulone). This hop has a strong citrusy profile, attributed to its high myrcene content, and confers a flavour profile of grapefruit, lime and tropical fruits to the beer (Hoplist, 2022a). In this product test, after boiling, an additional 5 L of grape wort were also added, with the goal to extract more flavour and have a more present taste of the grape wort in the final product.

Alcoholic fermentation with *Saccharomyces cerevisiae* (US-05) then occurred as usual, with the exception of a high krausen addition of hops. US-05 is an American ale yeast that produces neutral ales, clean and crispy. This yeast forms a firm foam head, with the ability to stay in suspension during fermentation. It is known for a production of a low amount of total esters, opposed to a higher content in higher alcohols. As for the attenuation, this yeast is capable of converting 78 % to 82 % of the fermentable sugars if kept at the ideal fermentation temperature of 18 °C to 26 °C, with an average flocculation and medium sedimentation. US-05 yeast presents an ethanol tolerance of 9 % to 11 % (Fermentis, 2022a) and the recommended dosage is 50 g/hL to 80 g/hL, being added at a concentration of 52.3 g/L in an initial phase of filling the fermentation vessel (so that hydration occurs at a higher wort temperature) through direct pitching, at the surface of the wort, progressively covering the surface in its entirety while avoiding clumps. The temperature designated for fermentation was 18 °C, although thermal control was difficult to achieve during this study.

Finally, the product was on rest, with forced carbonation implemented after the maturation process. The CO_2 levels intended were 2.6 g/L, and were chosen based on the style of beer and characteristics intended, in this case, higher levels of CO_2 to match and potentiate the refreshing aspect of the grape must. As for the adjuncts added, Go-Ferm Protect, a yeast autolysate, produced through a specific autolysis process, helps achieve high levels of certain essential vitamins (thiamine, pantothenic acid, biotin), minerals (magnesium, zinc and manganese), amino acids and lipids (unsaturated fatty acids and sterols) (Lallemand Wine, 2022). It is a product that aims to protect the yeast in difficult fermentation conditions, presented in this assay. DAP, or diammonium phosphate is a traditional source of nitrogen to aid yeast and help reduce the risk of problems associated with hydrogen sulphide. Besides these, $CaCO_3$ and Citric acid were used for the increase and decrease in wort pH, respectively.

Figure 6 presents the fermentation profile of this product test, a grape ale kettle sour (specific day data in appendix B, Table B1). More water had to be used than the previously expected for the mash thickness planned, as there would be no conditions to carry out mashing with the initial amount of water. To balance this in terms of ethanol content, less sparge water had to be used which comes with the downside of less fermentable sugars extracted in this process. The kettle souring was implemented at day 0 and ended at day 4, where the target pH was reached. Below that, the alcoholic fermentation would have significantly more difficulties to proceed. At this point, the product was boiled to eliminate the bacteria used for lactic fermentation and more grape wort was added, which was responsible for the increase in *ºPlato* at day 5. This kettle souring had the objective of conferring complexity to the final product through the addition of a sour taste. Citra hops were also added through dry hopping at day 12, with the goal of obtaining a more aromatic final product. Fermentation stopped rather early, at a *ºPlato* of 6.9 which corresponds to an attenuation of 46 %, significantly lower than the expected 78 % to 82 %. This could have been caused by a crossing of the alcohol tolerance threshold of the yeast. US-05 has the aforementioned attenuation rate below ethanol percentages of 9 % to 11 %, however the finished product had an *ABV* (alcohol by volume) of 7.2 % which comes close to the lower limit of the yeast's tolerance. This percentage of alcohol is higher than the expected, which may be a consequence of the storage of the grape wort, which with time, slowly suffers from fermentation from wild strains of yeast. Additionally, the low attenuation could have been a result of the acidity provided by the kettle sour, or other unforeseen human errors caused during the production process of this product.

The final product presented a dark brown colour with a high degree of turbidity and as for its aroma, the grape wort was slightly present, along with caramel notes and the citric aroma of the hop used. In terms of flavour a strong acidity at the beginning was then followed by a sweet aftertaste and the carbonation

process added a more refreshing side to the product. The complexity added by the cara aroma malt does not add much to a grape-based beer, serving more as a masking agent, rather than a potentiator to the grape must, and was an element to remove in the following product test.

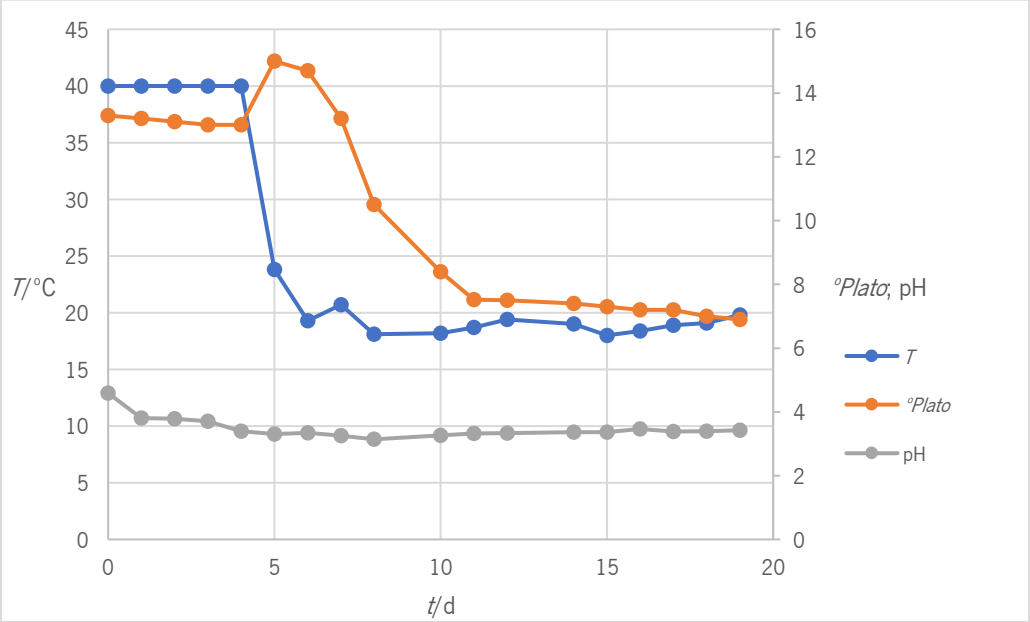

Figure 6. Variation of temperature (T), extract concentration ($^{o}Plato$) and pH during fermentation time (t).

Additionally, it was found that a lager base would prove beneficial due to the cleaner aromatic profile of the yeast. The acidity present was considered a positive point in this product, adding a very rich complexity that contrasts very well with the sweetness (which is an element to maintain) that comes after it, coming at the cost of masking the grape must, which implies that a reduction in this acidity is necessary to reach the aim of this work, which is to obtain a grape-based beer with a noticeable addition of grape must. Therefore, the kettle souring was also a process to remove, as the addition of grape must already provides a certain level of acidity, which was evaluated in the second product test. As for the hops used, the citric aroma was considered to complement the grape must aroma, being an element to maintain. However, the dry hop was perhaps unnecessary, as the citric aroma was more prevalent in the final product then the grape wort, being beneficial testing its absence in the following product test. The CO_2 levels chosen for the carbonation process conferred a more refreshing aspect to the product, which in a beer mashed with grape must was considered positive.

The sensory analysis trials conducted pointed to a not very successful product overall, with a global appreciation of 3 in a scale of 1-5 as seen in Figure 7, and on a consumer's point of view, very negative results, as out of 15 answers, 73.3 % of panellists voted "No" when inquired on the intent to buy.

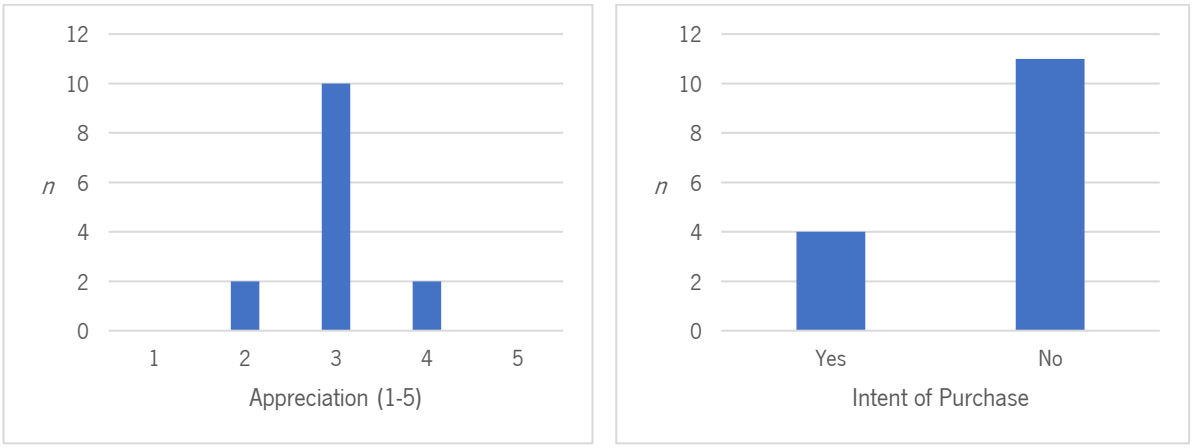

Figure 7. Global appreciation and intent of purchase of the Grape Ale beer based on number of answers (*n*).

Out of the characteristics evaluated in Figure 8 there were some positive conclusions, with the appearance and aroma of the beer being positively assessed, with 46.6 % and 40.0 % of panellists, respectively, rating a 4 in the used preference scale. These results go accordingly with the planned objectives, and in the most part mirrored the preliminary tasting that occurred by the time of production. However, from the point of view of the consumer, it appears that a grape-based beer would beneficiate from a higher bitterness than the one utilized for this product test, which differs from the conclusions and plans drawn by the author of the study.

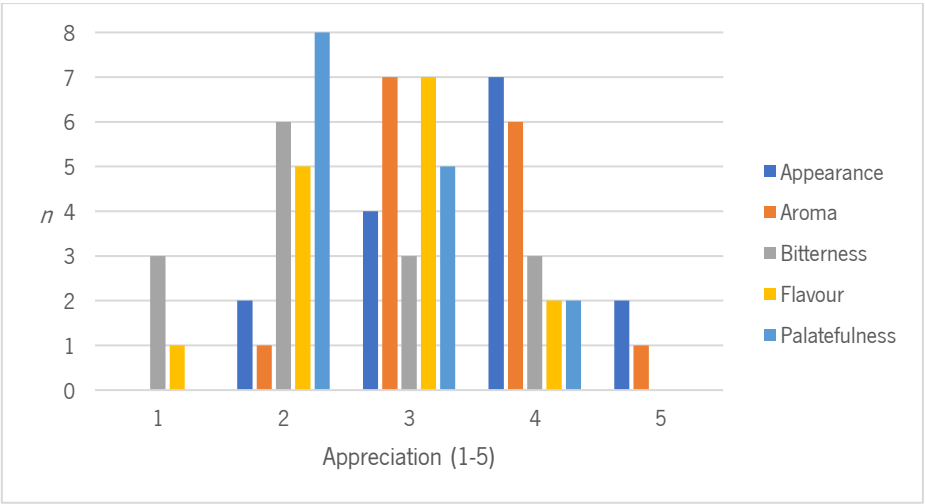

Figure 8. Sensory analysis regarding appearance, aroma, bitterness, flavour and palatefulness of the Grape Ale beer based on number of answers (*n*).

4.1.2. Second Product Test – Grape Lager

The following step was testing a bottom-fermented beer, which in light of the metabolism of the picked yeast, W-34/70, should produce a cleaner and more neutral beer, on where an addition of the same amount of grape wort as the previous product test should not go unnoticed. The chosen yeast is a lager yeast known for producing neutral beers with a good balance of floral and fruity aromas, that gives clean flavours and highly drinkable beers. It presents a low production of esters and higher alcohols with an apparent attenuation of 80 % to 84 % if kept at a temperature range of 12 °C to 18 °C. W-34/70 yeast has a fast sedimentation time, an alcohol tolerance of 9 % to 11 % and recommended dosage is 80 g/hL to 120 g/hL (Fermentis 2022b). This yeast was added at a concentration of 52.3 g/L which could explain the slow start for the fermentation, and the fermenting product was kept at 12 °C with the assistance of a cooler.

In addition to the designated lager malt, an unmalted cereal was also used, flaked oats to add texture and mostly, to create a certain degree of turbidity that could be an interesting feature for a grape must related product. The Flaked Oats used are an unmalted product containing high levels of lipids, β-glucans and gums, which impart a silky mouthfeel and creaminess to beer. The more Flaked Oats in the grist, the greater the effect, but upon passing 20 % of the grist, the β-glucans and gums would have a slowing effect on wort run off (Simpsons Malt, 2022b).

As for the mashing guidelines, the wort went through three stages in the ramp up of temperature, starting at 64 °C for 30 min, then 68 °C also for 30 min, and finally, a mash out at 75 °C (10 min). These ramps were designated in order to maintain the sweet aftertaste felt in the first product test, as at 68 °C α-amylase is at its optimal temperature activity, meaning that a higher conversion of starch into maltose, maltotriose and dextrin exists, the last being a non-fermentable sugar by the yeast. As seen with the first assay, a higher volume of water had to be used to enable mashing, which was then deducted in sparging. The grape must was added in its entirety (43.5 % of mashing volume) to the boiling wort, during the final 15 min of this stage, as to sterilize it and rid it of potential dangers to the finished product, but still maintain characteristics inherent to its volatile compounds. No dry hop was utilized for this product test to better check if this fermentation type suited the grape wort, and if its taste and aroma was noticeable on the finished product. However, the same type of hop was added during boiling since the citric aroma was enjoyable in the first product test (a low amount is used in order to have a product with low bitterness, since it could make a complex final product in conjunction with the acidity provided by the grape must).

Despite the slow start fermentation went mostly as expected, as seen in Figure 9 (specific day data in appendix B, Table B2), but suffered some setbacks close to its ending, as it stopped at 6.8. This

corresponds to an apparent attenuation of 46.6 %, significantly lower than the supposed 80 % to 84 % this yeast is capable of. These results could be a consequence of the low dosage of yeast used, the 68 °C mashing, or a lack of nutrients such as nitrogen for the yeast metabolism. Another explanation could be the presence of the grape wort, or even a high ethanol content. The W-34/70 yeast has an alcohol tolerance of 9 % to 11 %, and perhaps due to the prolonged storage of the grape must (at cold temperatures, which only slows down fermentation by wild strains of yeasts and bacteria) the *ABV* of this beer was 7.5 %, higher than expected even though fermentation did not convert all the fermentable sugars. Priming was achieved with the same conditions as the previous beer, as the refreshing aspect was something to maintain.

The final product presented a gold colour, with an interesting turbidity, not common on a lager beer. As for its aroma, malt and grape must were noticeable, with also citric hints. The grape must was also slightly present on the flavour of the finished product, especially on the aftertaste. The citric element provided by the Citra hops was present at very low levels, and since it did not mask the grape must, a dry hopping could be employed in following product tests. Given that fermentation ended at a high *°Plato*, the expected sweetness is also present which is not necessarily a negative point since it creates a pleasant balance together with the acidity from the grape must.

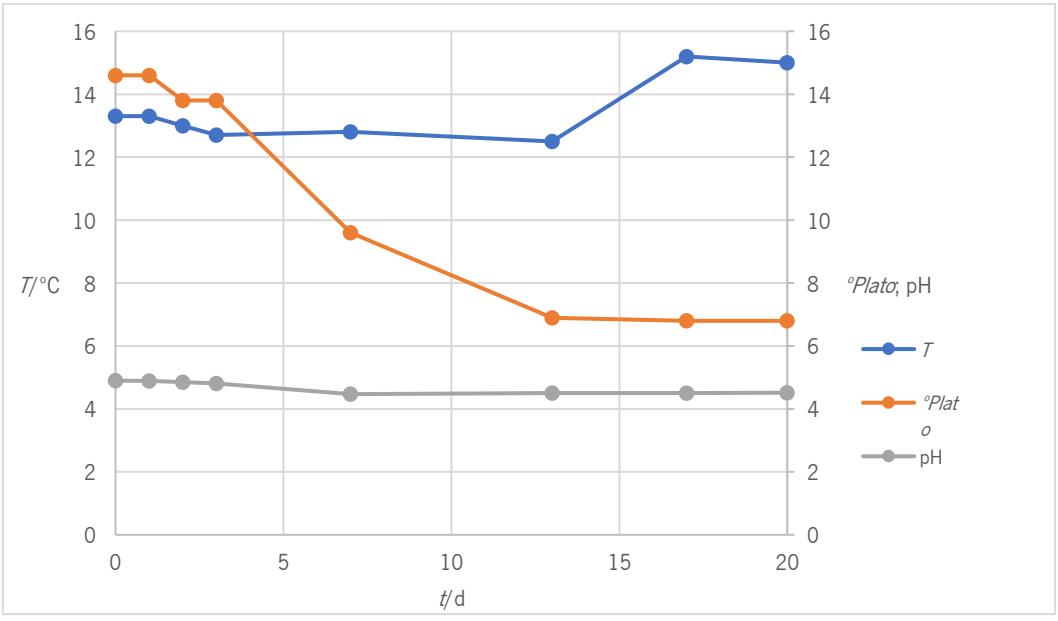

Figure 9. Variation of temperature (*T*), extract concentration (*°Plato*) and pH during fermentation time (*t*).

The *ABV* of 7.5 % gives this product a fuller body and heavier mouthfeel which was not something expected, but since the conditions of the grape wort could not be fully controlled and it already underwent a certain degree of fermentation, it was an aspect that had to be present for the next product tests. Overall,

the bottom fermentation proved to be very beneficial for the addition of grape wort, as a more neutral base made way for the grape must to be present in both the taste and aroma.

This product performed better overall than the other tests in terms of the consumer's opinion, which is highly reflected by the 53.3 % of panellists that corresponded to a 4 in the hedonic scale used for the global appreciation and the 80.0 % of panellists that voted "Yes" when inquired about intent to buy this product. An interesting result is the 20.0 % of panellists that graded 2 on global appreciation, but then again, this only reflects the fact that a grape-based product has a niche place on the market and is still very unknown to the public eye (Figure 10). As for the other categories appraised, most had very positive results, and the bitterness was the category where panellists were most divided (Figure 11), perhaps the most unexpected, as during production a low bitterness was found to be positive for this product, conclusions not shared by the panellists. As for flavour and aroma, perhaps the most significant of the appraised categories, the grape lager was highly ranked amongst the panellists, with a majority of answers grading 4 in the used scale.

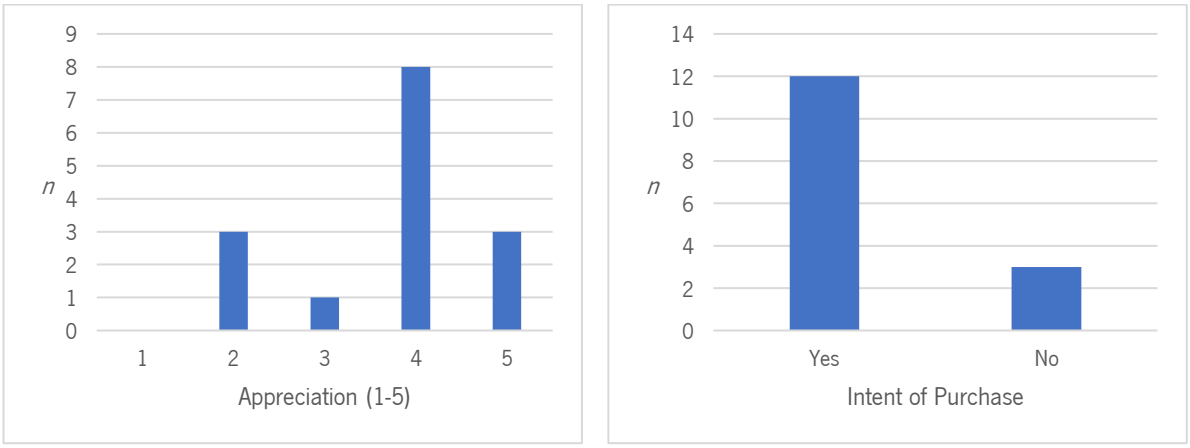

Figure 10. Global appreciation and intent of purchase of the Grape Lager beer based on number of answers (*n*).

When asked for descriptors on this particular product, only one participant attributed vinic notes to the beer, which implies that on the larger consumer view that are unaware of the production process, it still is hard to detect the grape must.

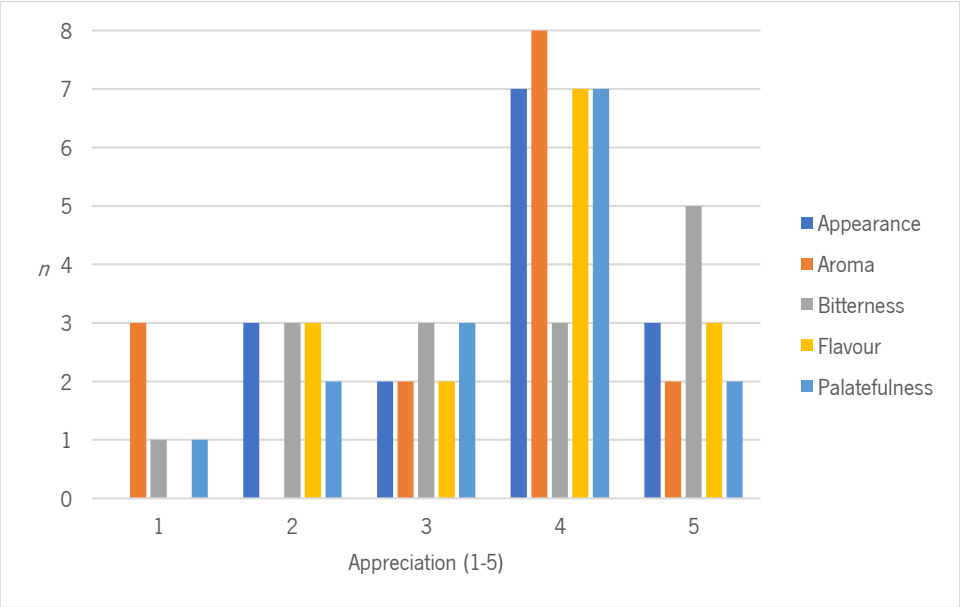

Figure 11. Sensory analysis regarding appearance, aroma, bitterness, flavour and palatefulness of the Grape Lager beer based on number of answers (*n*).

4.1.3. Third Product Test – Grape Weiss

The following fermentation type tested was, again, a top fermented product, but, that of a Weissbier, which is known for a very different aromatic profile from other beers, on account of the metabolism of the yeasts generally used for these beers, which can convert ferulic acid from the malt to 4-vinylguaiacol, responsible for a clove aroma and flavour (McMurrough, Madigan *et al.* 1996), and isoamyl alcohol to isoamyl acetate through the activity of an isoamyl alcohol acetyltransferase (Lilly, Lambrechts *et al.* 2000), the substance that gives beer banana flavour and aroma. Malts used included a lager malt and the fundamental wheat malt which meant that a mashing rest at 55 °C was necessary for the activity of β-glucanases. Wheat malt (Simpsons Malt, 2022c) can be added at 5 % to most Light Beers to increase foam stability without affecting haze or flavour and used at up to 70 % as a base malt for Wheat Beer, it imparts a bready flavour and characteristic citrus notes.

The mashing started at 55°C for 15 min, with a following rest at 64 °C for 45 min, and finally, a mash out at 76 °C. This assay showed the least inconsistencies with water volume used for mashing and sparging, as the planned amount was barely enough (only an extra 2 L were added). Since Weissbiers are usually known for their phenolic and fruity ester components, citric hops were deemed unnecessary and a hop more commonly present amongst wheat beers was used, Tettnanger, albeit at still a low quantity, as a low bitterness was wanted. This hop has a significantly high farnesene content which gives it a soft spiciness and a subtle, balanced, floral and herbal aroma. It is also great as a dual-use hop (Hoplist, 2022b). Grape must was added at the same stage as in the previous product test and in the

same amount. The yeast used, T-58, is a highly phenolic yeast generally used for its strong fermentation character, intense fruity and phenolic flavours, such as those of banana, clove and pepper. Suitable for wheat-base beers, this yeast produces a high quantity of esters and medium for higher alcohols as a result of its metabolism. It has an apparent attenuation of 72 % to 78 % if kept at the temperature range designated by the manufacturer of 18 °C to 26 °C. T-58 yeast also presents an alcohol tolerance of 9 % to 11 % and a medium sedimentation time. Recommended dosage is 50 g/hL to 80 g/hL (Fermentis, 2022c) and the planned concentration was 104 g/hL.

This product test differs from the previous in the use of Servomyces, a naturally enriched biological yeast nutrient, substituting the Go-Ferm nutrient. The propagation and drying process of this brewing yeast has been specifically designed to accumulate a range of trace minerals and elements that are essential or limiting during alcoholic fermentation. This yeast nutrient decreases fermentation time significantly, improves yeast sedimentation, stimulates uptake of maltose and maltotriose (higher alcohol levels), stimulates protein synthesis and yeast growth (higher biomass production during propagation), and helps eliminate harsh sulphur notes to produce a smoother and more balanced beer (Lallemand Brewing, 2022b).

As shown in Figure 12 (Table B3 in Appendix B), fermentation stopped once again early, at a *°Plato* of 6.81, at which point 500 mL of T-58 yeast in high activity from another fermentation were inoculated to attempt a restart, however, the *°Plato* only lowered to 6.62, which implies an apparent attenuation of 59.6 %. This result is still higher than the previous ones which could disprove the theory that the growing alcohol percentage of the grape must could be influencing negatively the fermentation process, however, other factors could have intervened, such as the lower original *°Plato*, more ideal pH for the yeast, or bigger supply of nutrients present such as nitrogen availability from the malts used. Still, the attenuation achieved was lower than expected since the T-58 yeast has an apparent attenuation of 72 % to 78 %, and the *ABV* for this beer was 6.9 %.

The finished product presented a pale gold colour with a cloudy turbidity common in wheat beers. The aroma was phenolic and slightly fruity, and the banana, or isoamyl acetate was more present on the flavour. Grape must was not quite traceable on either the aroma or the flavour, as the aromatic profile of this fermentation was considered not very suiting for the objective of this study. The strong phenolic and ester character provided from the yeast gave conflicting results as the clove and banana aroma conflicted with the grape must aroma. For the flavour, the same results were obtained. These results do not particularly imply a failed product as the resulting aroma and flavour was interesting, however, do not go

according to the objective of this study which was to see what fermentation style and other characteristics allowed for the addition of grape must to be well noted on the finished product.

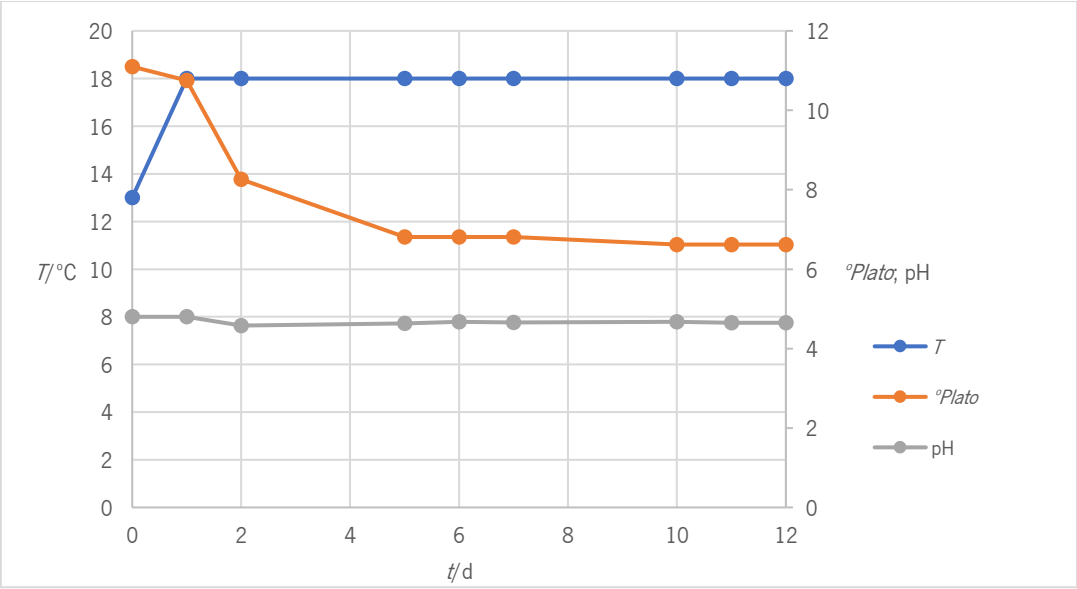

Figure 12. Variation of temperature (*T*), extract concentration (*°Plato*) and pH during fermentation time (*t*).

In terms of the sensory analysis trials results, this product test scored very similarly to the first product test, albeit to a more positive side. Appearance and aroma were the categories that were graded the best, with 60.0 % and 53.3 % of panellists to give a 4 out of 5 on the scale used, respectively as seen in Figure 13.

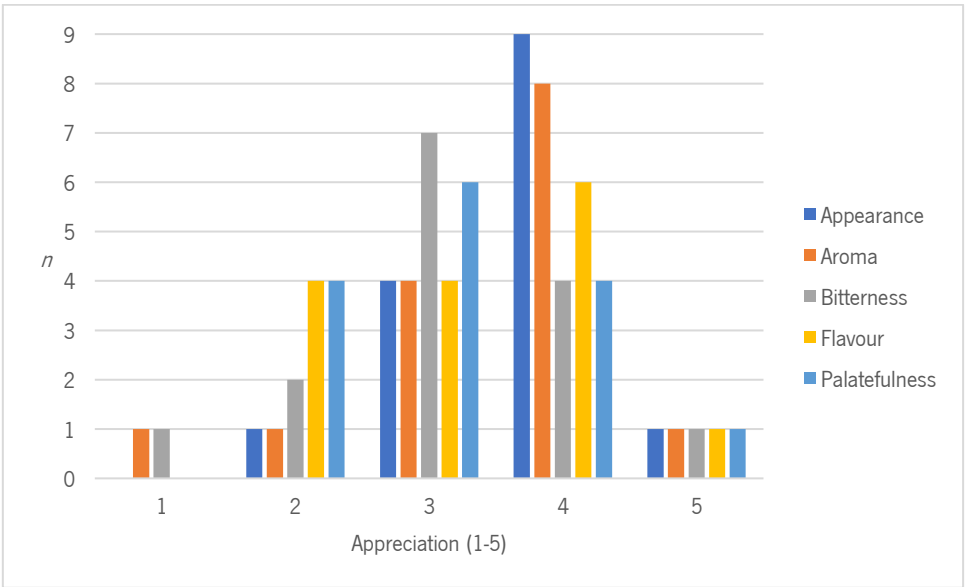

Figure 13. Sensory analysis regarding appearance, aroma, bitterness, flavour and palatefulness of the Grape Weiss beer based on the number of answers (*n*).

The aroma was found by the panellists to be pleasant with most attributing it a 4, which was not similar to the preliminary analysis conducted during production, as the phenolic and ester character was considered to conflict with the grape must. However, it is not very surprising that it ranked high with the panellist which evaluated the aroma during a blind test, where the clove and banana aroma seemed predominant and pleasant. Besides the aroma, bitterness was also perceived differently by the consumer and found to not be quite right. On the intention of purchase panellists were almost evenly divided with 46.6 % of "No" and 53.3 % of "Yes" (Figure 14).

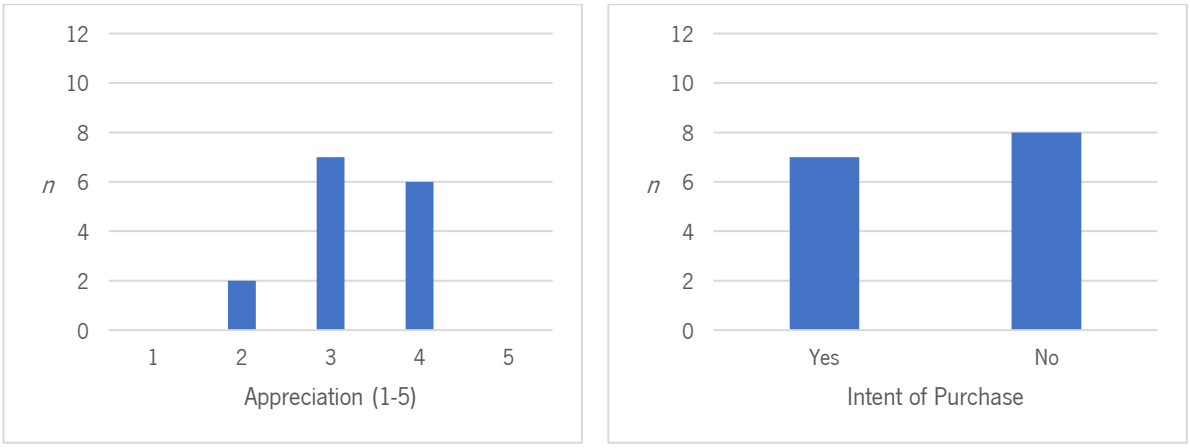

Figure 14. Global appreciation and intent of purchase of the Grape Weiss beer based on number of answers (*n*).

4.2. Culmination of the study – Final Product Test

According to the ideas that were gathered throughout this study and the production of the first three product tests, a final test was designed, following the characteristics that were considered successful from all the product tests. The main point, the fermentation style, was chosen from the second product test, which was the one where the addition of grape must was most noticeable. The W-34/70 yeast was used, at a concentration of 104 g/hL. Malts used were also meant to be kept from that product (amounts were increased for a higher final volume, but the rest of the conditions were adjusted so that the *ABV* could be similar) but flaked oats were not available and thus substituted with flaked wheat, which also confers turbidity and creaminess to the final product. Flaked Wheat is therefore an unmalted product that gives great foam stability and mouthfeel. This unmalted wheat product is packed with high molecular weight protein, which help give foam stability, as well as greater mouthfeel and turbidity, being traditionally used for head retention and to supplement wort nitrogen (Simpsons Malt, 2022d). The hop chosen was Citra, at a low quantity for a low bitterness, and two dry hopping additions were done, one during the highest activity of the QA23 yeast, high krausen, and the second before lowering the

temperature of the product for maturation. The QA23 yeast was used in an attempt to restart fermentation, and it is a yeast that is typically used for wines where fruit expression is desired, as it has a high β-glucosidase activity, which helps cleave non-volatile aromatic compounds into their volatile state. As for its oenological properties, it has an average lag phase and vigorous fermentation, low relative nitrogen demand, very low H_2S production, alcohol tolerance of 16 %, low SO_2 production, fermentation temperature (14 °C to 28 °C), competitive killer factor present, malolactic-bacteria compatibility and low foam production (Lallemand Brewing, 2022c).

Higher volumes of water had to be used for mashing, a common trend observed amongst these product tests, which implied no sparge water as the volume limits of the equipment were reached. Fermentation again stopped early, at *°Plato* of 7.6 as seen in Figure 15 (and Table B4, appendix B), which corresponds to an attenuation of 53 %. In the case of this product, besides the other already stated reasons for the fermentation to stop such as the growing alcoholic percentage of the grape must, problems subsisted throughout the process with the temperature, as it was impossible to maintain at the supposed 12 °C ideal for the yeast used (W-34/70). Instead, the temperature was unstable and varied between 13 °C and 20 °C which is not adequate to the yeast metabolism. Perhaps due to these reasons a sulphur-like aroma was detected nearly the end of the fermentation and measures had to be taken so that the finished product did not present it. For this, the product underwent copper treatment, and a removal of this off-flavour was successful. Before this treatment, an attempt at restarting the fermentation occurred with the starter of wine yeast QA23 which has a higher alcohol tolerance than W-34/70, however, this attempt was unsuccessful.

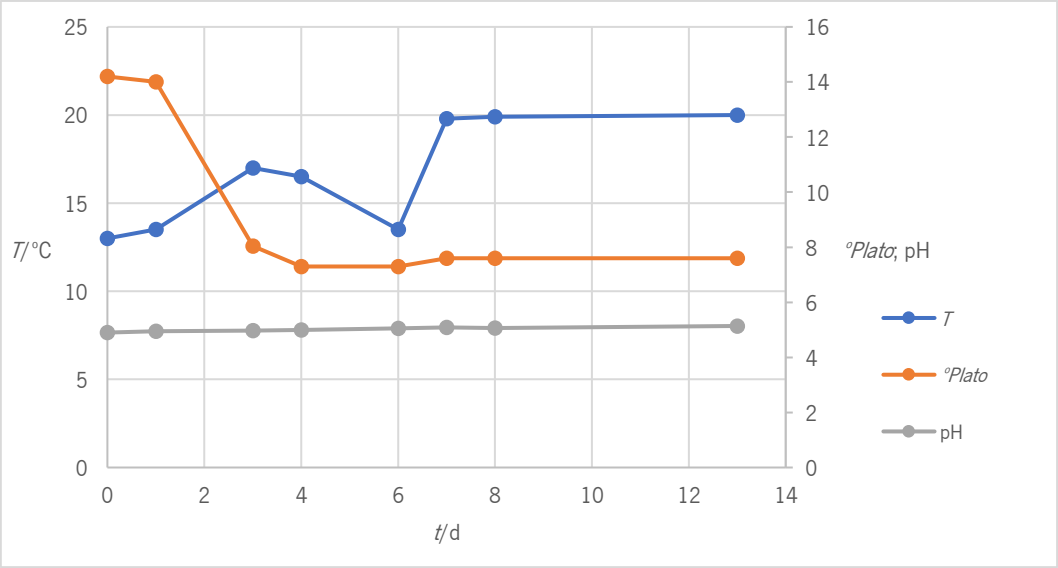

Figure 15. Variation of temperature (*T*), extract concentration (*°Plato*) and pH during fermentation time (*t*).

The finished product presented an appearance alike to the second product test, as the raw materials in its basis are very similar. As for the aroma, as a result of the dry hop additions of the Citra hop there was a very pleasant citric aroma that complements the grape must aroma. The flavour however was where this product failed, as due to the problems that surfaced during fermentation a high alcoholic content could be detected, and a strong sweetness was present. The grape must was nowhere to be found in the flavour, as the predominant flavour was sugary and alcoholic. As a consequence of this, the mouthfeel was also off, and the product presented a very heavy body. Unfortunately, unlike the first and third product tests that still had very positive points and could be enjoyed by certain consumers, this one should not. Since the objective of this product test was to finalize and optimize the formula created in the second product test, this product test failed on the aspect of flavour, and should be repeated in future when all conditions and requirements can be met, mainly those of the grape must, which means that this product is ideal in the short period following vintage, when the grape must is the freshest. Of course, other factors played in why this product test failed, but this one should be considered vital when attempting to perfect a formula for a grape-based beer.

The final product test scored relatively lower on all categories, having the highest percentage of panellists that voted "No" on intent of purchase (86.6 %) as seen in Figure 16.

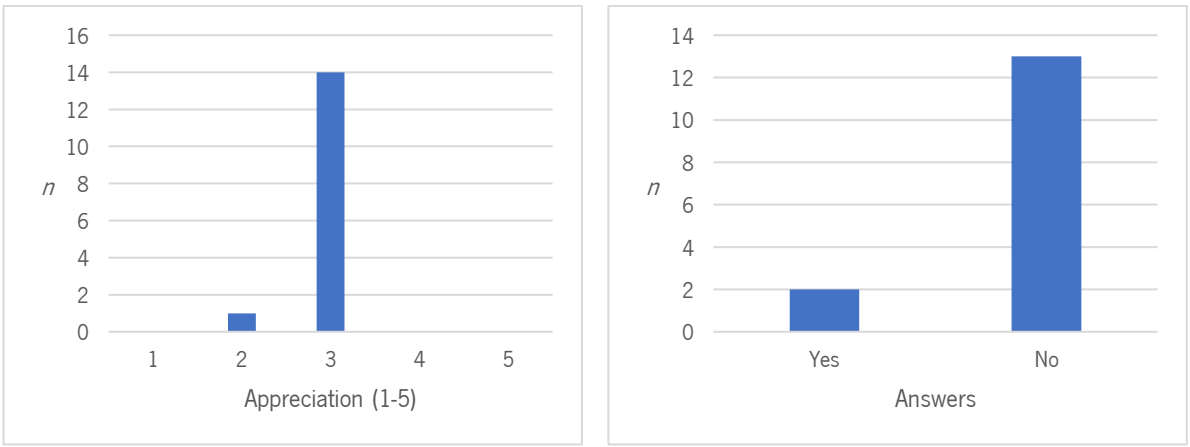

Figure 16. Global appreciation and intent of purchase of the final product test beer based on the number of answers (*n*).

Unlike the other products, this attempt however scored more positively on the bitterness (Figure 16) which is an unexpected result as a low amount of bittering hops was used like in the other product tests, and therefore a low bitterness was also present on the final product. Perhaps this could be due to a more confusing flavour and mouthfeel that altered the panellists' perception of bitterness or at least difficulted the consumer's ability to perceive the bitterness and may not mean that this product has a better

bitterness necessarily. No panellists corresponded to a 4 or 5 in the hedonic scale on the global appreciation which reflects the negative results of this product, and 93.3 % evaluated this beer with a 3.

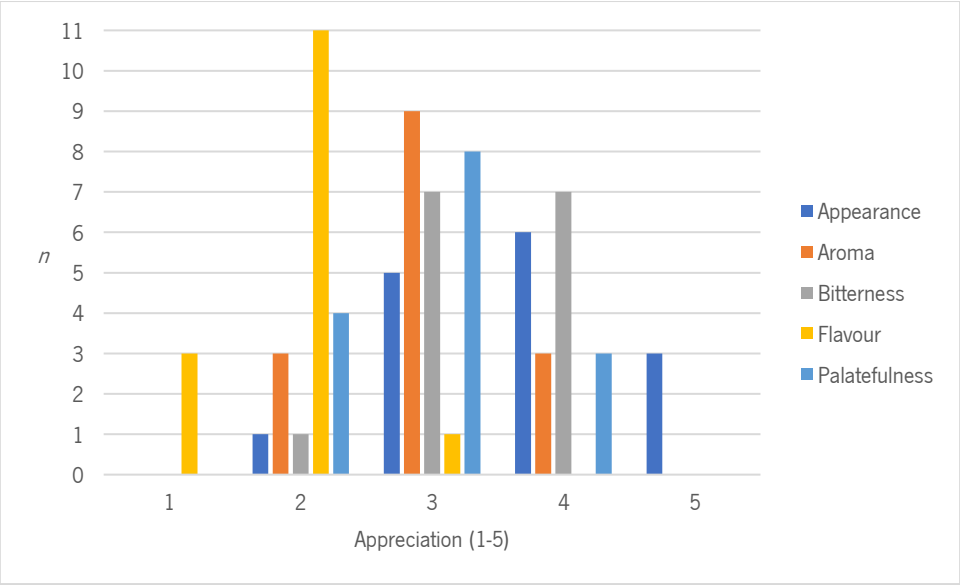

Figure 17. Sensory analysis regarding appearance, aroma, bitterness, flavour and palatefulness of the final product based on the number of answers (*n*).

4.3. Sensory Comparison of the Beers Produced in the Product Tests

The results obtained of the acceptance test described on the methods and materials section are hereby summarized, where the responses were averaged for a radar graphic which provides better visualization (Figure 18).

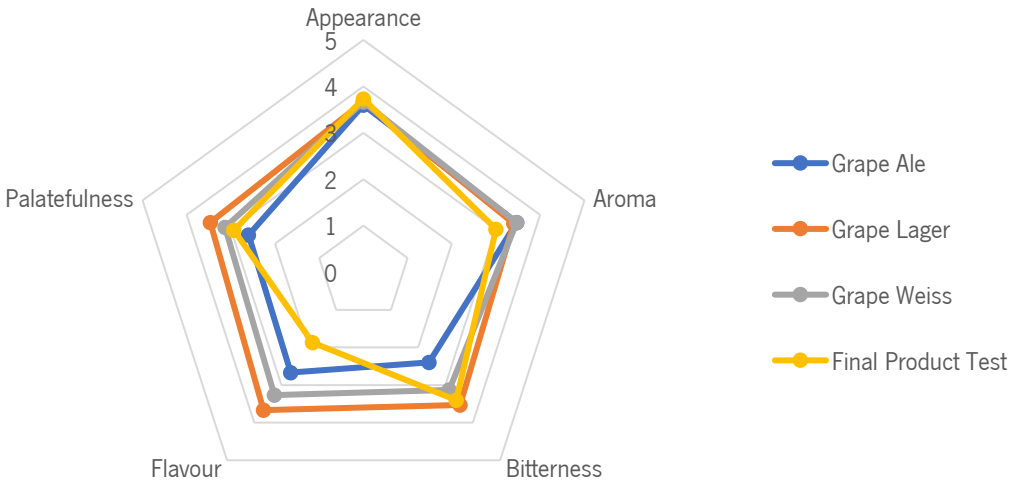

Figure 18. Sensory analysis results for appearance, aroma, bitterness, flavour and palatefulness.

Regarding global appreciation, which is here defined as the average of the scores on all the previous categories given to a specific product test by one panellist, the results obtained were used for a statistical comparison of the four product tests.

Using this data of 15 panellists on the global appreciation of the four products, the sum, average, standard deviation and 95 % confidence interval were determined using Excel (Table C1, Appendix C). The obtained test statistic (Table C2, Appendix C) was found to be higher than the tabulated value, and therefore, a significant difference between the four products exists.

For the post-hoc analysis using Fisher's LSD test, the tabulated values in Table C3 (Appendix C) for the degrees of freedom for error and significance level was used to check for differences between the products when compared two-by-two. The LSD_{rank} calculated was 13.86, and to determine if there were significant differences, the absolute value of the difference between the sum of global appreciation of the two products was calculated (Table C4, Appendix C). According to the Fisher's LSD test as a post-hoc analysis of the Friedman's test, if the absolute value of the difference between the sum of global appreciation of the two products is superior to the LSD_{rank} obtained, then the two products are here considered significantly different. Using this assumption, only two sets of products were concluded to be significantly different, the first and second product tests, and second and final product tests. These results go according to the assessments made during production as the second product test stood out from the first and final product tests. An interesting and not expected at all result is the similarities between the first and final product tests, as the final should by all means be considered a failed attempt and worst on the consumer's eyes to the first product test. However, since the sample tested was only of 15 people, and since the first product test is an even more niche version of an already niche product (sour grape-based beer), these results could be suffering of a lack of variety among the consumers that served as panellists, which means that in the future, if an industrial scale of this product is to be attempted, a bigger sample for sensory analysis trials is required. As for a more graphical representation of the results of the global appreciation sensory analysis represented in Table D1, Appendix D, a box and whisker plot proves beneficial (Figure 19), and for such, the median, minimum value, first quartile, third quartile and maximum value were determined (Table C5, Appendix C).

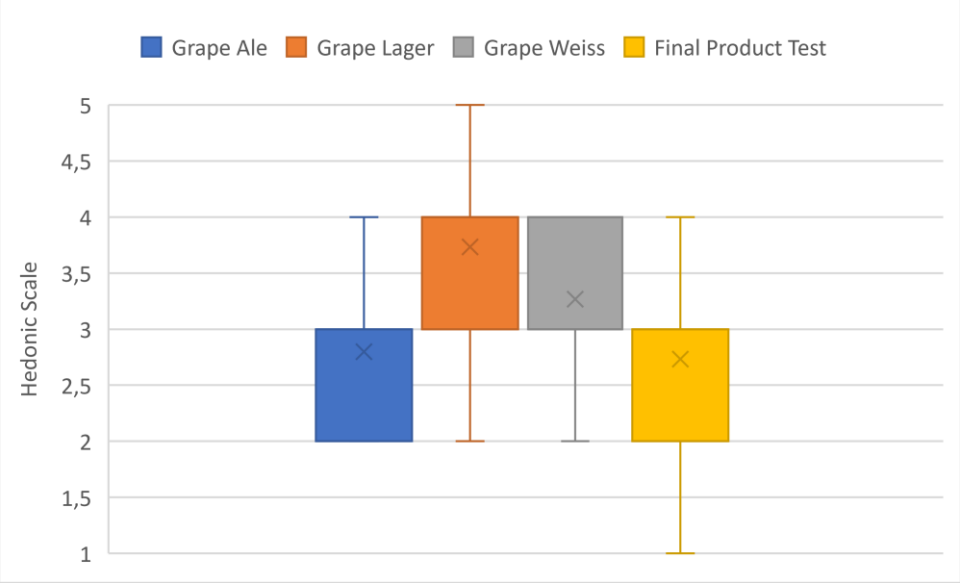

Figure 19. Box and whisker plot of the sensory analysis results regarding global appreciation.

4.4. Future Tests

In sight of the results obtained future testing would be beneficial for the product given the innovative character of a product that is already inserted in an innovative area of products, craft beer. However, careful planning is necessary for the product in question since the grape must is required to be kept at low rates of fermentation in order to not introduce additional variables, and in order to fully ferment the sugars from the grape must with the intended yeast, instead of wild strains. The positive elements from the final product test should be kept in future tests, namely the type of fermentation (bottom-fermented) and hop utilized, as well as the dry hopping technique which produced notable results. With a more established basis of this product, which was attempted in the final product test but impossible to achieve due to the conservation status of the grape must or other factors, future tests can focus on adding elements to this basis such as fruits, or more specialized malts, with the ending result being a more unique product that plays on ingredients that enhance the wine characteristics of this beer, so that it may reach a more niche part of the market. For the sensory analysis aspect of this study, more conclusions and insight on the viability of these products could be attained from using a wider sample of panellists, as well as more varied in social context, as the majority of the participants were a part of the brewing industry.

4.5. Industrial Plan

The product development tests were incorporated in an innovation plan at the factory at hand, with what could be considered a research and development laboratory. As part of this process, smaller quantities are produced, in experimental lots, which can be worked on and further modified to a final version that is fit to be produced in a larger scale batch. For that, the recipe and production process have to be adapted to the industrial quantities and available equipment, and as such, an industrial plan has been drafted, to which an HACCP approach is beneficial, in order to ensure product safety and quality.

Based on the seven principles of HACCP, a food safety plan was drafted for the product at hand, based on the HACCP plan established at Sovina Lda, where these product trials were conducted. For the production of a grape-based craft beer the process is first initiated by receiving the raw materials, grape must, malts, hops and yeasts with different types of storage required. Following this step, the production of beer can begin, with the cereals and water being loaded onto the mash tun with quantities that follow the specification of each recipe. A recirculation of must occurs after mash out, and the resulting product goes through lautering. Following lautering, the wort is sent back to the mash tun and boiled, where hops are introduced at the times specified by the recipe. Some adjuncts can be added during mashing such as citric acid or calcium carbonate to control the pH if necessary, and salts which are required at the factory at hand due to utilization of reverse osmosis water. After boiling, an addition of yeast nutrients can occur if deemed necessary. Before being sent to the fermenter the wort is cooled through a plate heat exchanger, and during fermentation daily analysis of pH and $^{o}Plato$ are required in order to monitor the process, which can help prevent the development of off flavours through contamination. This process is described by the flow chart in Figure 20.

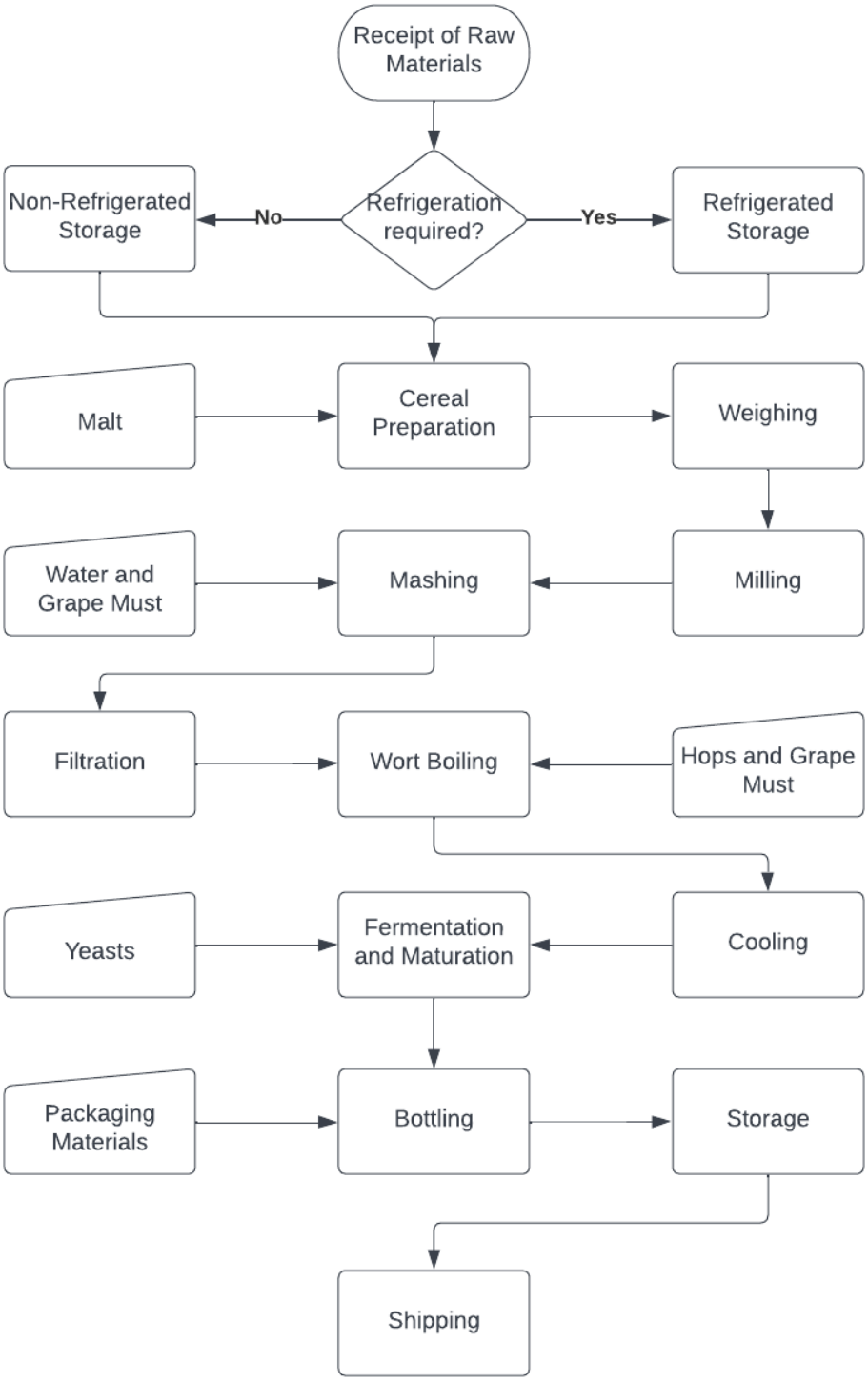

Figure 20. Beer production flow chart.

If additional processes such as the kettle souring and dry hopping addition are required, the flow chart on Figure 21 applies.

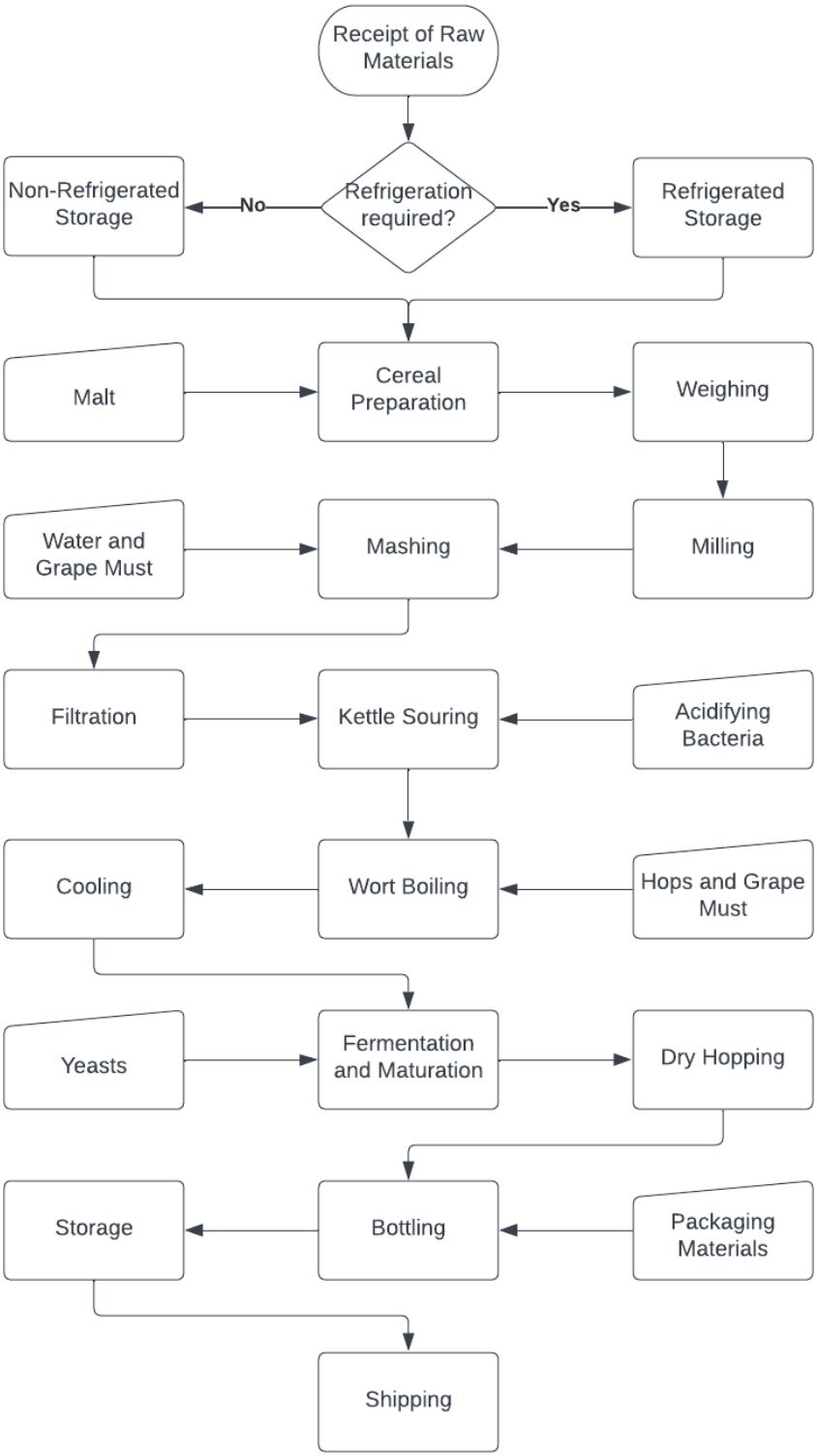

Figure 21. Beer production flow chart when kettle souring and dry hopping are in place.

Based on the first principle of HACCP, all possible hazards were analysed through a 4x4 risk assessment matrix (Table 4) and are listed for sequential and systematic analysis so that significative hazards are identified, in Table 5. For the risk assessment matrix drafted significative hazards were defined as having

a risk value of four or higher, as to prevent against dangers which have a high probability of occurrence but low severity, and also, dangers with high severity of occurrence but low probability.

The probability of occurrence criteria subdivides into four categories, high (4), where the presence of the identified hazard is certain, being always present, medium-high (3), in which case the presence of the hazard is common, being regularly detected (at least once a year) or the normal practices of the activity can lead to its occurrence, medium (2), when the presence of danger is possible, having occurred in the past on some sporadic situation or there is history or bibliography that supports its presence, and, finally, low (1), where the occurrence is practically impossible or there is minimal presence of danger, having not existed in the past.

Table 4. Risk assessment matrix

Probability					
	High (4)	4	8	12	16
	Medium-High (3)	3	6	9	12
	Medium (2)	2	4	6	8
	Low (1)	1	2	3	4
		Low (1)	Medium (2)	Medium-High (3)	High (4)
				Severity	

As for the severity of occurrence, high (4) implies serious health effects, requiring prolonged hospitalization and/or death, medium-high (3) where the effects lead to the affected individual having to be hospitalized, medium (2), in which the effects can be reversed by medical attention, rarely does the affected individual have to be hospitalized, and lastly, low (1), which implies mild severity, and may cause indisposition or discomfort.

Based on the 2nd principle, the installation of the HACCP plan makes it possible to identify the stages in which the risk is sufficiently high and in which it will be necessary to use control methods and reduce risk levels to acceptable levels, promoting the implementation of a processing line with controllable risk (Table 5). To identify these CCPs (critical control points), a Decision Tree is used (Figure 22), which, through a sequence of questions about the possible hazards that may occur, allows the detection of the steps where it is necessary to carry out a control in order to reduce or eliminate these same dangers.

Table 5. Hazard identification and risk analysis throughout the grape beer production and bottling process

Stage	Type of Hazard	Hazard	Risk			Control Measures
			P	S	R	
Receipt of raw materials	B	Presence of mycotoxins or microbial growth	1	3	3	Visual inspection of packages (fungi, moulds, pests, insects).
	Q	Presence of heavy metal residues or pesticides	1	2	2	Analysis of heavy metals and pesticides report sent by the supplier for each batch.
	F	Presence of foreign bodies	1	1	1	Visual inspection of packages.
Raw material storage	B	Microbial growth	1	3	3	Have a stock rotation procedure in place, compliance with hygiene plan, pest control plan and storage procedures.
	F	Presence of pests and insects	1	2	2	Have a stock rotation procedure in place, compliance with hygiene plan, pest control plan and storage procedures.
	F	Cross contamination	1	1	1	Compliance with hygiene plan and storage procedures.
Milling	F	Cross contamination	3	1	3	Compliance with hygiene plan for the equipment and enforce frequent hand hygiene.
Brewing	B	Presence of insects	1	2	2	Visual inspection and compliance with cleaning and sanitation plan.
	F	Presence of foreign bodies	1	1	1	Visual inspection and compliance with cleaning and sanitation plan.
Addition of water	B	Presence of pathogenic microorganisms	1	3	3	Compliance with analysis plan.
	Q	Presence of heavy metals	1	2	2	Reverse osmosis filtration.
Boiling	B	Survival of pathogenic microorganisms	1	4	4	Compliance with manufacturing practices and the hygiene plan for equipment and utensils; control of boiling time and temperature.
	F	Presence of foreign bodies	1	2	2	Compliance with manufacturing practices in place, during utilization of equipment. Visual inspection of ingredients added.
Addition of grape must	B	Presence of pathogenic microorganisms	1	3	3	Regular laboratory analysis and compliance with addition timings.

Table 5. Hazard identification and risk analysis throughout the grape beer production and bottling process (cont.)

Stage	Type of Hazard	Hazard	Risk			Control Measures
			P	S	R	
Cooling	Q	Cooling chemical contaminants	1	4	4	Compliance with manufacturing practices, visual inspection of equipment, and training employees.
Fermentation	B	Presence of wild yeasts and/or bacteria	1	3	3	Compliance with cleaning and sanitation plan.
	Q	Chemical contaminants (waste from detergents and sanitizers)	1	3	3	Compliance with cleaning and sanitation plan.
Dry Hopping	B	Contamination of beer in fermenter that induces later microbial growth	1	3	3	Compliance with cleaning and sanitation plan as well as good hygiene practices.
	F	Introduction of foreign bodies	1	2	2	Compliance with good manufacturing practices.
Filtration	B	Non-conforming or unsuitable filter	1	1	1	Training employees, compliance with cleaning and sanitation plans.
	F	Filter not compliant. Presence of foreign particles	1	1	1	Training employees, compliance with cleaning and sanitation plans and careful supplier selection.
Bottling	B	Unsanitized bottles that can cause post-filling contamination	1	3	3	Compliance with cleaning and sanitation plan.
	F	Presence of foreign bodies (glass), defective bottles (fractures, chips)	1	4	4	Training employees, compliance with manufacturing practices and work instructions, and careful visual inspection.
Shipment (palettizing)	F	Presence of glass	1	1	1	Visual inspection
Finished Product Storage	Q	Organoleptic changes	3	1	3	Comply with the storage plan (humidity, light, temperature and bottle position)

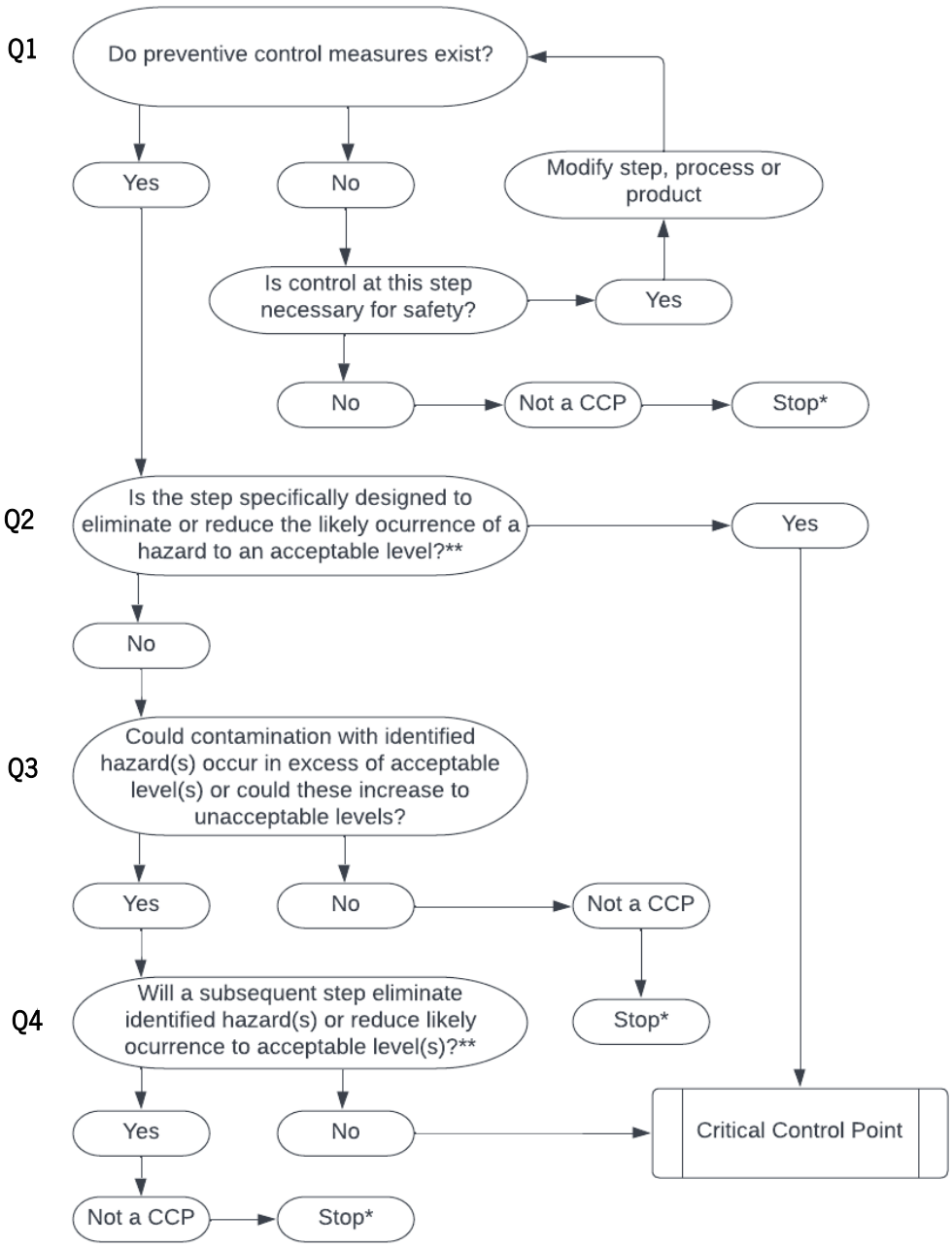

FIGURE 22. HACCP DECISION TREE FOR THE IDENTIFICATION OF CCPS

With the aid of the Decision Tree, several critical control points were identified, and are described in Table 6. Next, it is crucial to define the critical limits for these steps, as well as establish monitoring procedures. Critical limits serve as a preventive measure associated with a CCP, corresponding to extreme parameters of acceptability in relation to the safety and quality of the product, being possible values to observe or measure. Each CCP stage will have its critical limits according to possible hazards

and control methods. Along with critical limits, it is necessary to define procedures for monitoring the control methods to ensure that the CCP steps are under control. These procedures have a determined frequency, as well as a specific person in charge of monitoring.

Following the establishment of critical limits and methods of monitoring these, it is necessary to implement corrective actions for when critical limits are exceeded. In this way, for each hazard of each CCP there must be a specific corrective action, in which the destination of the product that does not comply with the limits of the CCP is indicated. All records must be documented and validated.

After establishing corrective actions, procedures for verifying the HACCP system applied must also be stipulated, in which the registration procedures and verification of the validation of the tests, methods and procedures adopted for the monitoring of the CCPs are verified. These verification procedures are performed using an external auditor and may include a review of the plan. In Table 7, the 3rd and 4th principles are applied, presenting critical limits and control methods associated with CCPs, as well as the corrective actions and verification procedures, obtained in accordance with the 5th and 6th HACCP principles. Finally, applying the 7th principle, it will be necessary to develop a system for recording the results of tests carried out for the designated CCPs for which it will be necessary that all documentation related to HACCP is compiled.

Table 6. Identification of critical control points throughout grape-based beer production and bottling process

Stage	Type of Hazard	Hazard	Q1	Q2	Q3	Q4	CCP?	Notes
Boiling	B	Survival of pathogenic microorganisms	S	N	N		N	
Cooling	Q	Cooling chemical contaminants	S	N	S	N	S	**CCP1**
Bottling	F	Presence of foreign bodies (glass), defective bottles (fractures, chips)	S	N	S	N	S	**CCP2**

Table 7. Establishment of critical limits, corrective measures, monitorization procedures, associated documents and person responsible

CCP	Stage	Critical Limits	Corrective Measures	Monitorization	Associated Documents	Responsible
CCP1	Cooling	Presence of chemical contaminants above permitted limit	Discard lots since last ok check of the critical limits, start production of new lots. Maintenance of equipment.	Measure of chemical contaminants through refractometer; Frequent substitution of cooling chemicals may imply leaks.		Production Manager and Brewer
CCP2	Bottling	Glass presence detected	Reject non-compliant bottles	Visual inspection		Brewer

5. Conclusions

The increasing demand of new and differentiated products on the foods and drinks industry gives space to an increase in research and development for the launch of new commercially viable products. One such sector of the drinks industry is the brewing industry, where craft breweries represent the forefront of innovation in terms of new products. Beers that impart characteristics from wine through the use of grape must are a possibility for innovation within the industry and the wine-context of Portugal.

With developing an innovative product within these characteristics in mind, the fermentation type that was deemed more suitable for a grape-based beer was the bottom-fermentation, or, a Lager beer where addition of grape must occurs at 50 % of mashing volume. This combination alongside the unmalted cereals for creaminess and citra hops for its aromatic profile make for a very clean base for a grape-based beer, where a better detection of grape must is allowed.

However, this is a delicate and complex product subject to adversities and difficult quality control, that is only optimal on a short period after vintage, as the grape must condition, in the amounts used in this study, heavily influences the final product. Other parameters that complicate production of beer with grape must are the difficult conditions it creates for yeast, namely the lower pH and higher alcohol content, which may cause several complications during fermentation. Planning around the nitrogen availability for the yeast is also a crucial factor for this product, and mashing rests should be well defined according to the attenuation level desired.

Sensory-wise, aroma and appearance were categories that evaluated positively on most product tests with the addition of grape must to 50 % of mashing volume, but the Grape Lager stood out among the panellists in terms of flavour, which then heavily influences global appreciation and intent of purchase. This beer was the most successful, however, the Grape Weiss also shed an interesting light on the acceptability of Weissbiers, as it draws on many characteristics from them.

The statistical analysis conducted pointed to similarities between the Grape Ale and final product test (derived from the Grape Lager), and a certain degree of statistical similarities between the Grape Lager and Grape Weiss. The ones where significant differences were detected were the pairs Grape Ale and Grape Lager, and Grape Lager and the final product test. Nevertheless, positive points were found on all product tests, which further help define a future for a grape-based beer.

References

Alfeo, V., Todaro, A., Migliore, G., Borsellino, V., Schimmenti, E., (2019). Microbreweries, brewpubs and beerfirms in the Sicilian craft beer industry. *International Journal of Wine Business Research*, 32(1), 122-138.

Alves, V., Gonçalves, J., Figueira, J. A., Ornelas, L. P., Branco, R. N., Câmara, J. S., & Pereira, J. A. (2020). Beer volatile fingerprinting at different brewing steps. *Food chemistry*, 326, 126856.

Andrews, J. (2006). The brewhouse. Chapter 10. In C. W. Bamforth (editor) Brewing: new technologies, Woodhead Publishing, pp. 208-227.

Aroh, K, Review: Beer Production. Retrieved from https://ssrn.com/abstract=3458983 or http://dx.doi.org/10.2139/ssrn.3458983 (accessed on October 2022)

Asare, E. K., Jaiswal, S., Maley, J., Baga, M., Sammynaiken, R., Rossnagel, B. G., & Chibbar, R. N. (2011). Barley grain constituents, starch composition, and structure affect starch in vitro enzymatic hydrolysis. *Journal of Agricultural and Food Chemistry*, 59(9), 4743-4754.

Bamforth, C. (2006). Brewing: new technologies: Woodhead Publishing.

Banco de Portugal (2021). Análise das empresas da indústria das bebidas. Retrieved from https://bpstat.bportugal.pt/conteudos/publicacoes/1340 (accessed on October 2022)

Baptista, P., Pinheiro, G., & Alves, P. (2003). Sistemas de gestão de segurança alimentar. Forvisão, Consultoria em Formação Integrada, Guimarães.

Bastgen, N., Becher, T., Drusch, S., & Titze, J. (2020). Usability and technological opportunities for a higher isomerization rate of α-acids: A Review. *Journal of the American Society of Brewing Chemists*, 79(1), 17-25.

Birch, R. M., Ciani, M., & Walker, G. M. (2003). Magnesium, calcium and fermentative metabolism in wine yeasts. *Journal of Wine Research*, 14(1), 3-15.

Bongaerts, D., De Roos, J., & De Vuyst, L. (2021). Technological and environmental features determine the uniqueness of the lambic beer microbiota and production process. *Applied and Environmental Microbiology*, 87(18), e00612-00621.

Brányik, T., Vicente, A. A., Dostálek, P., & Teixeira, J. A. (2005). Continuous beer fermentation using immobilized yeast cell bioreactor systems. *Biotechnology progress*, 21(3), 653-663.

Briggs, D. E. (1978). Barle. Springer Science & Business Media.

Briggs, D. E. (1998). Malts and malting. Springer Science & Business Media.

Brown, S., Oliver, S., Harrison, D., & Righelato, R. (1981). Ethanol inhibition of yeast growth and fermentation: differences in the magnitude and complexity of the effect. *European Journal of Applied Microbiology and Biotechnology*, 11(3), 151-155.

Capece, A., Romaniello, R., Siesto, G., & Romano, P. (2018). Conventional and non-conventional yeasts in beer production. *Fermentation*, 4(2), 38.

Costell, E. (2002). A comparison of sensory methods in quality control. *Food Quality and Preference* 13(6), 341-353.

Marin, A. C., Baris, F., Romanini, E., Lambri, M., Montevecchi, G., & Chinnici, F. (2021). Physico-chemical and sensory characterization of a fruit beer obtained with the addition of cv. Lambrusco grapes must. *Beverages*, 7(2), 34.

Conway, J. (2021a). *Statista.* Beer production worldwide from 1998 to 2020. Retrieved from https://www.statista.com/ (accessed on October 2022),

Conway, J. (2021b). *Statista.* Global leading countries in beer production 2020. Retrieved from https://www.statista.com/ (accessed on October 2022).

Conway, J. (2021c). *Statista.* Volume of beer produced annually in Portugal from 2008 to 2019. Retrieved from https://www.statista.com/ (accessed on October 2022).

Conway, J. (2021d). *Statista.* The world's leading 10 brewing groups in 2020, based on production volume. Retrieved from https://www.statista.com/ (accessed on October 2022).

Cooper, R. G. (1990). Stage-gate systems: a new tool for managing new products. *Business Horizons*, 33(3), 44-54.

Cooper, R. G. (2008). Perspective: The stage-gate® idea-to-launch process—update, what's new, and nexgen systems. *Journal of Product Innovation Management*, 25(3), 213-232.

de Mello, E. B., Ganzer, P. P., Rasia, I. C. R. B., Olea, P. M., & da Rocha, J. M. (2012). Processo de desenvolvimento de produtos e o sistema Stage-Gate. *Gestão Contemporânea*, edição especial, 117-137.

de Francesco, G., Marconi, O., Sileoni, V., Perretti, G. (2021). Barley malt wort and grape must blending to produce a new kind of fermented beverage: A physicochemical composition and sensory survey of commercial products. *Journal of Food Composition and Analysis*, 103, 104-112.

de Roos, J., & de Vuyst, L. (2019). Microbial acidification, alcoholization, and aroma production during spontaneous lambic beer production. *Journal of the Science of Food and Agriculture*, 99(1), 25-38.

de Roos, J., & de Vuyst, L. (2022). Lambic beer, a unique blend of tradition and good microorganisms. In F. J. de Bruijn (editors) Good Microbes in Medicine, Food Production, Biotechnology, Bioremediation, and Agriculture, pp. 225-235.

Denault, L. J., Glenister, P., & Chau, S. (1981). Enzymology of the mashing step during beer production. *Journal of the American Society of Brewing Chemists*, 39(2), 46-52.

Dysvik, A., Liland, K. H., Myhrer, K. S., Westereng, B., Rukke, E. O., De Rouck, G., & Wicklund, T. (2019). Pre‐fermentation with lactic acid bacteria in sour beer production. *Journal of the Institute of Brewing*, 125(3), 342-356.

Fermentis (2022a). SafAle™ US-05. Retrieved from https://fermentis.com/en/product/safale-us-05/ (accessed on August 2022)

Fermentis (2022b). SafLager™ W-34/70. Retrieved from https://fermentis.com/en/product/saflager-w-34-70/ (accessed on August 2022)

Fermentis (2022c). SafAle™ T-58. Retrieved from https://fermentis.com/en/product/safale-t-58/ (accessed on August 2022)

Fuller, G. W. (2016). New food product development: from concept to marketplace. CRC Press.

Garavaglia, C. (2018). The birth and diffusion of craft breweries in Italy. In C. Garavaglia (editor) Economic Perspectives on Craft Beer, Springer, pp. 229-258.

Garavaglia, C. (2020). The emergence of Italian craft breweries and the development of their local identity. In C. Garavaglia (editor) The geography of beer, Springer, pp. 135-147.

Golob, T., Jamnik, M., Bertoncelj, J. (2005). Sensory analysis: methods and assessors. *Agris*, 85(1), 55-66.

Grönlund, J., Sjödin, D. R., & Frishammar, J. (2010). Open innovation and the stage-gate process: A revised model for new product development. *California Management Review*, 52(3), 106-131.

Habschied, K., Mastanjević, K. (2022). Maintaining the Quality Control of Beer. In: Glavaš, H., Hadzima-Nyarko, M., Karakašić, M., Ademović, N., Avdaković, S. (editors) 30th International Conference on Organization and Technology of Maintenance (OTO 2021). OTO 2021. Lecture Notes in Networks and Systems, vol 369. Springer.

Hai, Z. C. (2011). The impact of water quality on beer fermentation. 2011 International Conference on New Technology of Agricultural, Zibo, China, 27-29 May, 2011, pp. 643-645.

Hoplist (2022a). Citra. Retrieved from https://www.hopslist.com/hops/dual-purpose-hops/citra/ (accessed on August 2022).

Hoplist (2022b). Tettnanger. Retrieved from https://www.hopslist.com/hops/aroma-hops/tettnang-tettnanger/ (accessed on August 2022).

Hough, J.S., Briggs, D.E., Stevens, R., Young, T.W. (1982). Yeast Growth. In Hough, J. S. (editors) Malting and Brewing Science. Springer.

INE – *Instituto Nacional de Estatística* (2021). Principais indicadores das Empresas por Localização geográfica (NUTS – 2013) e Atividade económica (Subclasse – CAE Rev. 3); Anual. https://www.ine.pt/

Køie, B., Ingversen, J., Andersen, A., Doll, H., & Eggum, B. (1976). Composition and nutritional quality of barley protein. *International Atomic Energy Agency* (IAEA), 8(8), pp. 55-61.

Kappler, S., Krahl, M., Geissinger, C., Becker, T., Krottenthaler, M., (2010). Degradation of iso-α-acids during wort boiling. *Journal of the Institute of Brewing* 116(4), 332-338.

Klose, C., Thiele, F., & Arendt, E. K. (2010). Changes in the protein profile of oats and barley during brewing and fermentation. *Journal of the American Society of Brewing Chemists*, 68(2), 119-124.

Kunze, W. (2004). Techonology Brewing and Malting 3rd ed., VLB Berlin.

Lallemand Brewing (2022a). WildBrew Helveticus Pitch™. Retrieved from https://www.lallemandbrewing.com/en/canada/product-details/wildbrew-helveticus-pitch/ (accessed on August 2022).

Lallemand Brewing (2022b). Servomyces. Retrieved from https://www.lallemandbrewing.com/en/canada/product-details/servomyces-d50/ (accessed on August 2022).

Lallemand Brewing (2022c). Lalvin QA23™. Retrieved from https://www.lallemandbrewing.com/en/canada/product-details/lalvin-qa23/ (accessed on August 2022).

Lallemand Wine (2022). Go-Ferm Protect™. Retrieved from https://www.lallemandwine.com/pt-pt/portugal/produtos/catalogue/nutrientes-e-protetores-do-vinho/4/goferm-protect/ (accessed on August 2022)

Lawless, H., Heymann, H. (2010). Acceptance Testing. In Lawless, H. (editors) Sensory Evaluation of Food, Food Science Text Series, Springer.

Likens, S., Nickerson, G., Haunold, A., & Zimmermann, C. (1978). Relationship Between Alpha Acids, Beta Acids, and Lupulin Content of Hops. *Crop Science*, 18(3), 380-386.

Lilly, M., Lambrechts, M., Pretorius, I. (2000). Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. *Applied and Environmental Microbiology,* 66(2), 744-753.

Machado, S. (2019). Craft beer in Portugal: a study about consumers, perceptions, drivers and barriers of consumption. Dissertação de Mestrado em Gestão, Universidade Católica Portuguesa.

Masschelein, C. A. (1986). The biochemistry of maturation. *Journal of the Institute of Brewing*, 92(3), 213-219.

McMurrough, I., *et al.* (1996). Control of ferulic acid and 4-vinylguaiacol in brewing. *Journal of the Institute of Brewing,* 102(5), 327-332.

McMurrough, I., Cleary, K., & Murray, F. (1986). Applications of high-performance liquid chromatography in the control of beer bitterness. *Journal of the American Society of Brewing Chemists*, 44(2), 101-108.

Meilgaard, M. (1960). Hop analysis, cohumulone factor and the bitterness of beer: review and critical evaluation. *Journal of the Institute of Brewing*, 66(1), 35-50.

Meilgaard, M., Carr, B., Civille, G., (2016). Sensory evaluation techniques, CRC Press.

Murray, J., Delahunty, C., Baxter, I., (2001). Descriptive sensory analysis: past, present and future. *Food Research International*, 34(6), 461-471.

Moir, M. (2000). Hops — a millennium review. *Journal of the American Society of Brewing Chemists*, 58(4), 131-146.

Montanari, L., Floridi, S., Marconi, O., Tironzelli, M., & Fantozzi, P. (2005). Effect of mashing procedures on brewing. *European Food Research and Technology*, 221(1), 175-179.

Mortimore, S., & Wallace, C. (2013). HACCP: A practical approach. Springer Science & Business Media.

Nance, M. R., & Setzer, W. N. (2011). Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing. *Journal of Brewing and Distilling*, 2(2), 16-22.

Nobile, M. D., D'Amato, D., Altieri, C., Corbo, M. R., & Sinigaglia, M. (2003). Modeling the yeast growth-cycle in a model wine system. *Journal of Food Science*, 68(6), 2080-2085.

Noël, S., Liégeois, C., Lermusieau, G., Bodart, E., Badot, C., & Collin, S. (1999). Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *Journal of Agricultural and Food Chemistry*, 47(10), 4323-4326.

Novais, M. d. R. (2006). Noções gerais de Higiene e Segurança Alimentar – Boas Práticas e Pré-Requisitos HACCP. *Segurança e Qualidade Alimentar*, 1, 10-11.

Ocvirk, M., & Košir, I. J. (2020). Dynamics of isomerization of hop alpha-acids and transition of hop essential oil components in beer. *Acta Chimica Slovenica*, 67(3), 720-728.

OECD – *Organization for Economic Co-operation and Development* (2005). Oslo Manual. Paris and Luxembourg: OECD/Euro-stat.

Oliveira, J. M. M. (2001). Aromas varietais e de fermentação determinantes da tipicidade das castas Loureiro e Alvarinho. Tese de Doutoramento em Engenharia Química e Biológica, Universidade do Minho.

O'Leary, R. (2008). Method of analysis for correcting dissolved CO_2 content for specific gravity and alcohol variations in beer. *BevSense LLC*, 1-4.

O'Rourke, T. (2002). The function of wort boiling. *Brewer International*, 2(2), 17-19.

Parker, D. (2012). Beer: Production, sensory characteristics and sensory analysis. In Piggott, J. (editor) Alcoholic beverages, Woodhead Publishing, pp. 133-158.

Penso, C. C. (2003). Modelo de referência para o processo de desenvolvimento de produtos na indústria de alimentos. Dissertação de Mestrado em Engenharia Mecânica, Universidade Federal de Santa Catarina, Brasil.

Pires, E., & Brányik, T. (2015). Biochemistry of beer fermentation, Springer.

Punčochářová, L., Pořízka, J., Diviš, P., & Štursa, V. (2019). Study of the influence of brewing water on selected analytes in beer. *Potravinarstvo Slovak Journal of Food Sciences*, 13(1), 507-514.

Pusecker, K., *et al*. (1999). Investigation of hop and beer bitter acids by coupling of high-performance liquid chromatography to nuclear magnetic resonance spectroscopy. *Journal of Chromatography A*, 836(2), 245-252.

Rieger, M., Käppeli, O., & Fiechter, A. (1983). The role of limited respiration in the incomplete oxidation of glucose by *Saccharomyces cerevisiae*. *Microbiology*, 129(3), 653-661.

Schneiderbanger, J., Grammer, M., Jacob, F., & Hutzler, M. (2018). Statistical evaluation of beer spoilage bacteria by real-time PCR analyses from 2010 to 2016. *Journal of the Institute of Brewing*, 124(2), 173-181.

Schönberger, C. (2009). Why cohumulone is better than its reputation. *Brauwelt International*, 27(3), 159-160.

Simpsons Malt (2022a). Finest Lager Malt. Retrieved from https://www.simpsonsmalt.co.uk/our-malts/finest-lager-malt/ (accessed on August 2022).

Simpsons Malt (2022b). Flaked Oats. Retrieved from https://www.simpsonsmalt.co.uk/our-malts/flaked-oats/ (accessed on August 2022).

Simpsons Malt (2022c). Wheat Malt. Retrieved from https://www.simpsonsmalt.co.uk/our-malts/wheat-malt/ (accessed on August 2022).

Simpsons Malt (2022d). Flaked Wheat. Retrieved from https://www.simpsonsmalt.co.uk/our-malts/flaked-wheat/ (accessed on August 2022).

Sovina. (2022). Sovina – Cerveja Artesanal. Retrieved from https://sovina.pt/ (accessed on October 2022)

Tarí, J. J., Molina-Azorín, J. F., & Heras, I. (2012). Benefits of the ISO 9001 and ISO 14001 standards: A literature review. *Journal of Industrial Engineering and Management*, 5(2), 297-322.

TBE – The Beers of Europe (2018). Beers Statistics. Retrieved from https://brewersofeurope.org/uploads/mycms-files/documents/publications/2018/EU-beer-statistics-2018-web.pdf (accessed on August 2022).

Tornai-Lehoczki, J., & Dlauchy, D. (2000). Delimination of brewing yeast strains using different molecular techniques. *International journal of food microbiology*, 62(1-2), 37-45.

Weyermann, 2022. Weyermann® CARAAROMA®. Retrieved from https://www.weyermann.de/en-gb/product/weyermann-caraaroma-8/ (08/2022).

White, C., & Zainasheff, J. (2010). Yeast: the practical guide to beer fermentation: Brewers Publications.

WHO. (2018). Global status report on alcohol and health 2018. Retrieved from https://www.who.int/ (2022).

Willaert, R. (2007). The beer brewing process: Wort production and beer. In Y. H. Hui (editor) Handbook of Food Products Manufacturing, John Wiley & Sons, pp. 443-506.

Worsfold, D. (2001). A guide to HACCP and function catering. *The Journal of the Royal Society for the Promotion of Health*, 121(4), 224-229.

Appendices

A – Raw Material Characteristics

In Table A1 the technical specifications from the manufacturer's website of the malts and unmalted cereals are present, and in Table A2 the technical specifications for the hops used in this study.

Table A1. Available technical specifications (from the manufacturer's website) for the malted and unmalted cereals used

	Finest Lager	Flaked Oats	Wheat Malt	Flaked Wheat
Moisture %	4.5	10 – 12	3.0 – 4.5	12.5
Extract lt°/kg (7Dry)	307	300	315	310
Colour °EBC	2.0 – 4.0	2.5 – 3.5	2.0 – 6.0	1.0 – 1.5
Total Nitrogen %	1.80	-	1.95	-
Total Soluble Nitrogen %	0.50 – 0.65	-	0.9	-
Soluble Nitrogen Ratio	36 – 41	-	36 – 42	-
Friability %	96	-	50 – 80	-
Soluble Extract Fine-Coarse Difference %	-	-	2 – 6	-
Homogeneity %	98	-	-	-
Glucan in Wort (ppm)	120	-	-	-
Diastatic Power IOB "Dry"	45 – 65	-	-	-
Screenings % <2.2mm	2	-	-	-
NDMA (ppb)	2.5	-	-	-
Sulphur Dioxide on Malt (mg/kg)	3	-	-	-

Table A2. Technical specifications for the Citra brand hop from Yakima Chief Hops and Tettnanger hop from Charles Faram

	Citra	Tettnanger
Characteristics	Citrus, grapefruit, lime, tropical fruits, harsh bitterness	Balanced floral and herbal aromas with some spiciness
Purpose	Bittering and aroma	Bittering and aroma
Alpha Acid Composition	12 % – 15 %	3 % – 5.8 %
Beta Acid Composition	3.0 % – 4.5 %	2.8 % – 5.3 %
Co-Humulone Composition	20 % – 24 %	24 %
Total Oil Composition	1.5 % – 3.0%	0.36 % – 1.07 %
B-Pinene	0.7 % – 1.0 %	-
Myrcene Oil Composition	60 % – 70 %	40.6 %
Linalool	0.6 % – 1.0 %	-
Humulene Oil Composition	7 % – 12 %	20.4 %
Caryophyllene Oil	5 % – 8 %	6.2 %
Geraniol	0.3 % – 0.5 %	-
Farnesene Oil	0.1 % – 1.0 %	11.3 %
Other	6.5 % – 26.3 %	-

B – Fermentation Profile of the Product Tests

Day specific values of temperature, extract concentration (*ºPlato*) and pH for the fermentation and maturation process of the four beers produced, the Grape Ale (Figure B1), Grape Lager (Figure B2), Grape Weiss (Figure B3) and the Final Product Test (Figure B4).

Table B1. Variation of temperature (*T*), *ºPlato* and pH during fermentation of the Grape Ale beer

Day	*T* / ºC	*ºPlato*	pH
0	40	13.3	4.59
1	40	13.2	3.81
2	40	13.1	3.79
3	40	13.0	3.71
4	40	13.0	3.39
5	23.8	15.0	3.30
6	19.3	14.7	3.34
7	20.7	13.2	3.25
8	18.1	10.5	3.14
10	18.2	8.4	3.26
11	18.7	7.52	3.32
12	19.4	7.5	3.33
14	19.0	7.4	3.36
15	18.0	7.3	3.36
16	18.4	7.2	3.47
17	18.9	7.2	3.38
18	19.1	7.0	3.39
19	19.8	6.9	3.42

Table B2. Variation of temperature (*T*), *ºPlato* and pH during fermentation of the Grape Lager beer

Days	*T* / ºC	*ºPlato*	pH
0	13.3	14.6	4.90
1	13.3	14.6	4.89
2	13.0	13.8	4.85
3	12.7	13.8	4.81
7	12.8	9.6	4.47
13	12.5	6.9	4.51
17	15.2	6.8	4.51
20	15.0	6.8	4.53

Table B3. Variation of temperature (*T*), *ºPlato* and pH during fermentation of the Grape Weiss beer

Days	*T* / ºC	*ºPlato*	pH
0	18	11.1	4.80
1	18	10.75	4.80
2	18	8.26	4.58
5	18	6.81	4.63
6	18	6.81	4.67
7	18	6.81	4.66
10	18	6.62	4.67
11	18	6.62	4.65
12	18	6.62	4.65

Table B4. Variation of temperature (*T*), *ºPlato* and pH during fermentation of the Final Product Test

Days	*T* / ºC	*ºPlato*	pH
0	13	14.2	4.9
1	13.5	14	4.95
3	17	8.04	4.97
4	16.5	7.3	5
6	13.5	7.3	5.05
7	19.8	7.6	5.09
8	19.9	7.6	5.07
13	20.0	7.6	5.14

C – Statistical Analysis Calculations and Data

Statistical calculations and values used to assess differences between products through the Friedman Test and post-hoc analysis Fisher's Least Significant Difference test.

Table C1. Sum, average, standard deviation (SD) and 95 % confidence intervals (95 % CI) for the statistical analysis using Friedman's test

	A	B	C	D
Sum	42	56	49	41
Average	2.8	3.7	3.3	2.7
SD	0.7	1.0	0.7	0.7
95 % CI	0.3	0.5	0.4	0.4

Table C2. Test statistic and tabulated test statistic value for the Friedman's test

T	134.28
$\chi^2\alpha,\ t-1$	7.81

Table C3. Tabulated values for the determination of the LSD_{rank}

1-α	$z(\alpha/2)$
90 %	1.645
95 %	1.96
99 %	2.576
99.9 %	3.291

TABLE C4. Absolute value of the difference between the sum of global appreciation of two products

	Value in module/absolute
A-B	14
A-C	7
A-D	1
B-C	7
B-D	15
C-D	8

Table C5. Median, minimum value, first quartile, third quartile and maximum value for the sensory analysis of the 15 panellists of global appreciation

	A	B	C	D
Median	3	4	3	3
Minimum value	2	2	2	1
First quartile	2	3.5	3	2.5
Third quartile	3	4	4	3
Maximum value	4	5	4	4

D – Data on Global Appreciation of the Different Beers Produced for this Study

Values of global appreciation, calculated as the average of the five categories assessed in the acceptance test, appearance, aroma, bitterness, flavour and palatefulness.

TABLE D1. Global appreciation sensory analysis results for the four product tests

Panellist	Grape Ale	Grape Lager	Grape Weiss	Final Product Test
1	2	4	4	3
2	3	3	3	2
3	3	4	4	3
4	3	2	2	1
5	3	4	4	3
6	3	4	3	3
7	2	2	2	2
8	4	2	3	2
9	4	4	4	3
10	3	4	4	4
11	3	4	4	3
12	2	5	3	3
13	2	4	3	3
14	2	5	3	3
15	3	5	3	3