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Effect of Formulation on Physico-chemical and Sensory Properties of Beer

Master´s thesis

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Declaration

I hereby declare that I have completed this master's thesis, titled "**Effect of Formulation on Physico-chemical and Sensory Properties of Beer**", without any assistance except for guidance from my supervisors. Additionally, I confirm that all the sources of information used in the thesis are professional literature and have been acknowledged and listed in the bibliography at the end of the thesis. As the author of this thesis, I also confirm that I have not violated any copyright laws in the process of creating it.

Prague, 16th April 2024 Brem SAM

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Effect of Formulation on Physico-chemical and Sensory Properties of Beer

Summary:

The brewing industry continues to evolve with advancements in technology and consumer preferences, necessitating comprehensive studies to understand the intricate interplay between operating conditions, formulation choices, and the resulting chemical composition and sensory properties of beer. This thesis investigates the multifaceted effects of operating conditions and formulation on beer, focusing on the utilization of five diverse adjuncts: orange skin, red grape, white grape, sugar, and honey. The research seeks to evaluate the impact of incorporating different varieties of dried grapes and musts into beer formulations. By systematically varying the types of adjuncts used, the study aims to discern their influence on key parameters such as flavor profile, color, and mouthfeel. Lastly, the focus shifts to selecting the optimal beer formulation that maximizes process efficiency while enhancing compositional attributes and sensory characteristics. Through sensory analysis and chemical profiling, the goal is to identify the formulation that best aligns with consumer preferences.

Keywords: Brewing, fermentation, grape must, adjuncts, sensory

1 Introduction

Beer, a beverage enjoyed worldwide for millennia, holds a prominent place among the array of consumed drinks. The market offers a diverse selection of beer types and styles, tailored to meet the evolving preferences of consumers who exhibit a growing interest in artisanal products (Siesto et al., 2023a). For many years, the brewing industry has utilized adjuncts such as corn, rice, unmalted barley, wheat starch, and grapes to supply fermentable carbohydrates for yeast. The incorporation of adjuncts alongside or instead of barley malt serves various purposes, including enhanced accessibility in local markets, sensory modification of the beer, and notably, cost reduction (Siesto et al., 2023a).

In particular, the use of fruits into beer production emerges as a prominent trend observed worldwide, evident in both local, small-scale craft breweries and industrial brewing operations, as well as in numerous recent research studies (Gasiński et al., 2022a). Beers crafted with fruit adjuncts exhibit a rich sensory profile characterized by well-blended and balanced fruity flavors. Fruit beer stands out as a highly appealing product offered by micro- and craft breweries, boasting intriguing attributes including a high concentration of polyphenols, particularly phenolic acids, as well as aromatic compounds and notable antioxidant capacity (Siesto et al., 2023a).

Although beer remains the most widely consumed alcoholic beverage globally, it remains a subject of ongoing research. The focus of these research endeavors encompasses various aspects, including enhancing foam stability, exploring beer aging processes, and innovating the development of non-traditional beer styles. This continuous exploration underscores the dynamic nature of the beer industry, driven by a quest for improvement and innovation across diverse fronts. In this sense, the study examines thier production methods, as well as the attained chemical, physicochemical, and sensory characteristics. (Paiva et al., 2021).

2 Scientific Hypothesis and Objectives

2.1 Hypothesis

- Adding different varieties of dried grapes and grape musts to beer will notably influence its sensory attributes and composition.
- Evaluating various beer formulations with dried grapes and musts will identify an optimal formulation that enhances processes, composition, and sensory properties compared to traditional beers.

2.2 Objectives

This study aims to investigate "the effect of operative conditions and formulation on beer chemical and sensory properties". The main objectives of this research are:

- To evaluate the effect of adding different varieties of dried grapes and musts to beer.
- To select the best beer formulation considering the effect on processes, composition, and sensory properties.

3 Literature Review

3.1 Beer

3.1.1 Ale Beer

Beer making or brewing requires the use of germinated barley (malt), hops, yeast, and water. In addition to barley malt, other starches and/or sugar-containing raw materials have functional roles, e.g., wheat, unmalted cereals called adjuncts (unmalted barley, unmalted wheat, corn, rice), starch flour, starch degradation products, and fermentable sugars (Belitz et al., 2009). Beer is the most-produced fermented beverage in the world, with the annual production exceeding 1.97 billion hectoliters a year. Presently, most of the produced beer is classified as ale and lager beer by a unique fermentation method and the types of yeast used in the process (Mertens et al., 2015). Ale yeasts are commonly called top fermenters as the former tend to float to the top of the vessel at the end of the fermentation and lager yeasts are bottom fermenters while the latter sediment to the bottom of the vessel (Monerawela et al., 2015). The practice of brewing ale originated during the Middle Ages, and the fermentation process typically takes place at temperatures ranging from 20–30 ˚C using ale yeasts, which are predominantly diploid and belong to a specific species *Saccharomyces cerevisiae* (Monerawela et al., 2015). Ale yeasts present flotation and can trap CO_2 bubbles to form a yeast 'head' at the top of fermentation vessels (Hiralal et al., 2014). These top-fermenting yeast species are the main microbial workhorse to produce ale beers, which cover beer styles such as stouts, pale ales, doubles, triples, and quadruples (Stewart, 2016). Brown ale is usually red or copper-colored rather than brown which has a milder flavor than the other types of ale beer (Granato et al., 2011).

3.1.2 Lager beer

Lager beer is the most consumed alcoholic beverage worldwide. Its brewing process is conducted by fermentation at low (8 to 15°C) temperatures and using *Saccharomyces pastorianus*, an interspecific hybrid between *Saccharomyces cerevisiae* and the cold-tolerant *Saccharomyces eubayanus* (Mertens et al., 2015). Lagers are fermented by *S. pastorianus* which combines the desirable fermentation characteristics of *S. cerevisiae* with its other parent, *S. eubayanus*, which has the cold tolerance. Pilsner beer is the most popular and generally known type of lager beer which is fermented at lower temperatures (5 to 15°C), followed by a period of cold storage (lagering). This traditional practice vital for the beer's characteristically clean

flavor and aroma (Dequin & Casaregola, 2011). Currently, two distinct genotypes, dubbed "Saaz" and "Frohberg" are the originated commercially used lager strains. Saaz-type *S. pastorianus yeasts* are allotriploids (3n), while Frohberg-type is allotetraploids (4n), and each type has a slightly different phenotype and fermentation characteristics. Moreover, Saaz-type strains produce lower concentrations of aroma compounds like ethyl acetate, isoamyl alcohol, and isoamyl acetate (IA) than the more aroma-rich Frohberg yeasts (Walther et al., 2014).

3.1.3 Lambic Beer

Lambic beers are one of the oldest types of beers still brewed and are spontaneously fermented for one to three years before bottling. Lambic beers are originally brewed in or near the Senne River valley, an area near Brussels, Belgium. The production of lambic beer normally takes place only during the colder months of the year (October to March), since cold nights are needed to lower the wort temperature to about 20°C in one night (Spitaels et al., 2014). The production process of acidic ales of the lambic type, a mixed fermentation is normally obtained through air inoculation of the wort in an open coolship, followed by fermentation and maturation in horizontal wooden barrels (oak or chestnut), in which fermentation is carried out by enterobacteria, yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) (De Roos & De Vuyst, 2018). The final product of lambic beer is a noncarbonated sour beer that principally serves as a base for gueuze or fruit lambic beers. The sour taste of the beer arises from the metabolic processes of different types of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) (Spitaels et al., 2014). In the USA, sour beers are currently attracting interest. In contrast with traditional Belgian lambic breweries that exclusively produce lambic beers, American coolship ales copy the lambic beer production method, and such beers are a seasonal product from craft breweries, by the uses of *Saccharomyces* spp., for the brewing of other types of beers in the American craft-brewing sector, are enriched in these brewery environments (Bokulich et al., 2012a).

3.2 Raw materials

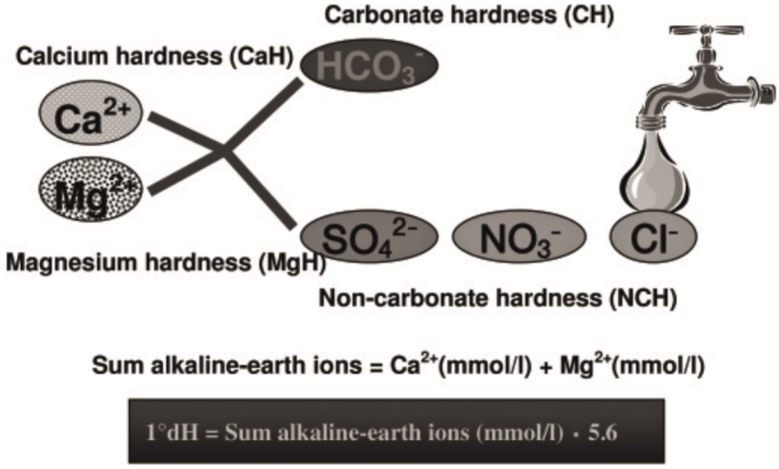
3.2.1 Brew Water

In terms of quantity, water is the most significant raw ingredient for beer. Since the brewing process is constituted of approximately 94% water, the chemical and biological content of water has substantial relevance in the manufacture of beer. As a result, water becomes a crucial, yet frequently abundant ingredient in the brewing process (Punčochářová et al., 2019). The production of beer involves the blending of the extracts of malt, hops, and sugar with water,

followed by its subsequent fermentation with yeast is energy-intensive and uses large volumes of water (Olajire, 2020). The water used for brewing needs to meet specific criteria in various areas. These criteria can be divided into "aesthetic" factors such as color, cloudiness, smell, and taste, as well as microbiological standards that require the absence of harmful bacteria. Additionally, the water's level of organic and inorganic substances that are dissolved and the presence of radioactive materials are also important factors to consider (Punčochářová et al., 2019).

Water hardness is an important factor in obtaining the quality of water used for brewing. The studies of brewing water composition generally involve the total hardness, which is defined as the sum of all alkaline-earth ions (calcium, magnesium, strontium, and barium ions). This hardness is divided into carbonate and non-carbonate hardness. The common counter ions for non-carbonate or permanent hardness are sulfate, nitrate, and chloride, and these remain in the solution when the water is boiled. Carbonate or temporary hardness is related mainly to calcium and magnesium bicarbonates and is so-called because if the water is boiled the bicarbonate is converted to carbonate, which causes leaving the clarified water “softened” (Briggs, 2004; Ziegler, 2007a). Soft water has a low number of dissolved salts, specifically calcium and magnesium salts. In contrast, hard water has high concentrations of salts, typically consisting mainly of calcium bicarbonate or calcium sulfate. It's essential to distinguish between the two, especially when using the water for mashing or sparging. This is because calcium and magnesium salts are the primary elements found in water (Briggs, 2004). Two additional crucial parameters that are closely linked to water hardness are pH and ionic strength. Furthermore, to meet legal requirements, other quality standards must be met when selecting water for brewing. This is because the ions present in water can impact the pH of the mash, wort, and beer, affecting both enzymatic and non-enzymatic reactions. As a result, they have a significant impact on acidity. Hydrogen carbonate ions are considered acid-neutralizing because they cause an increase in pH. Conversely, calcium and magnesium ions are acid-supporting and cause a decrease in the mash's pH (Ziegler, 2007b).

Figure 1 shows that carbonate and non-carbonate hardness are two types of total hardness. Nitrate, chloride, and hydrogen carbonate are common counter ions for carbonate hardness and sulfate for non-carbonate hardness (Ziegler, 2007).



(Ziegler,2007)

Figure 1 *The hardness portion of water*

Table 1 shows the quality criteria of brew water including pH, total hardness, residual alkalinity, and chemical composition (sulfate, chloride, nitrate, iron, and free aggressive of CO_2) (Ziegler, 2007).

Table 1 Quality criteria of brew water

Characteristic	Value	Reason
pH	7-8	too acidic: danger pf corrosion; too basic inhibition of enzymes
p	0-0.3 mval/L	Water does not contain aggressive CO2, but only low fraction of CO^{2-}_3; and OH^-
m	0.7-1.2 mval/L	only low residual of acidic destroying HCO^-_3; low fraction but positive for palatable taste
Non-carbonate hardness	at least twice, better three times the carbonate hardness	Balance alkalinity
Residual alkalinity	-2 to 2°dH <5 °dH <10 °dH	for Pilsner beers for light beers for dark beers
Sulfate	100-150 mg/L	dry bitterness, tendency to a hop aroma
Chloride	<100 mg/L	salty taste, corrosion
Nitrate	<25 mg/L	Fermentation disturbances are avoided; low val better as nitrate is also introduced into the beer hop and malt.
Iron	<0.1 mg/L	flaws in taste, danger of gushing and turbidity, and beer taste in stability
Free aggressive CO_2	-	Danger of corrosion

3.2.2 Yeast

Yeast is cultured in an acidic aqueous sugary solution called wort prepared from barley malt and other cereals such as maize, wheat, rice, sorghum, and also cane and beet sugar. Brewer's yeast cultures largely come from the genus Saccharomyces and a minority of non-Saccharomyces yeast cultures (Stewart, 2016). Brewing yeasts are divided into two classes, ale yeast, and lager yeast. Ale beer is brewed by domesticated strains of *Saccharomyces cerevisiae* at relatively high temperatures (15°C–26°C), while lager beer is generally brewed by the yeast *S. pastorianus* at lower temperatures (5°C–15°C) (Magalhães et al., 2016). Brewing yeasts have a great influence on the beer process and quality, with specific types being used to produce particular brewing styles. Although different groups vary in different properties, they generally share characteristic domestication traits such as strong flocculation, efficient sugar use, and lack of off-flavor (Cubillos et al., 2019). To make energy and gain material for reproduction, yeast converts sugar into chemical compounds such as alcohol, carbon dioxide, and other compounds that influence the taste of fermented foods and beverages. Yeast feeds on sugars to create alcohol, yet the source of sugars and their complexity will result in different fermentation conditions. The types of sugars created in the mash, present in malt extract, or added to the kettle or fermentor affect the fermentability of the wort (White & Zainasheff, 2010). These microorganisms play a crucial role in the brewing industry because they have the ability to ferment diverse substrates, resulting in the production of ethanol and secondary metabolites. In nature, there are over 1,000 different yeast species identified and many more strains. *Saccharomyces cerevisiae* is the best-characterized yeast, and it is also the most common top-fermentation brewer's yeast. The hybrid between *S. cerevisiae* and *S. bayunus*, *S. pastorianus* is the most common bottom-fermenting lager yeast.

S. cerevisiae is the yeast species that dominates in the production of alcoholic beverages worldwide, and the strains of this species employed in fermentation exert a profound influence on the flavor and aroma characteristics of different beverages (Walker & Stewart, 2016). *S. cerevisiae* is also the most common top-fermentation brewer's yeast, but the variation of this species is large. The different strains are carefully trialed and chosen for their specific characteristics in the fermentation of a brew made from grain and hops into a specific brand of beer (Linda, 2009). In industrial beer fermentations, some *S. cerevisiae* strains, can produce an extracellular glucoamylase enzyme that enables fermentation on oligosaccharides and starch if present. They are able to release glucose from the non-reducing end of any residual oligosaccharides, which in turn leads to extended fermentation if these diastatic strains

contaminate beer. This can have numerous various effects on the beer, such as increased carbon dioxide and ethanol levels, drier mouthfeel, and production of off-flavor, particularly the clove-like 4-vinyl guaiacol. This can also lead to gushing and exploding packages, endangering the consumer in extreme cases. In contrast, diastatic strains of *S. cerevisiae* are important spoilage microbes. (Krogerus & Gibson, 2018).

Saccharomyces pastorianus is an interspecies hybrid yeast involving *S. cerevisiae* and *S. eubayanus*, becoming one of the world's most important industrial brewing organisms. This yeast is used in lager-style beer production which the fermentation requires a very low temperature (8 to 15°C)(B. Gibson et al., 2014). Wort fermentation by *S. pastorianus* strains is normally started off by a fast, preferential consumption of glucose and fructose. While most *S. pastorianus* strains have a strong ability for maltose fermentation, the speed of maltotriose fermentation is typically greatly slower, resulting in extended fermentation process times and, in many cases, incomplete fermentation of this sugar. (Gänzle et al., 2017).

Table 2 shows several differences in ale and lager beer production, one of the major ones being the characteristics of the ale and lager yeast strains employed and the fermentation temperatures (Stewart, 2016).

Table 2 Differences between ale and lager yeast strains

Ale Yeast	Lager Yeast
Saccharomyces cerevisiae (ale type)	*Saccharomyces pastrorianus*
Typical fermentation temperature 18-25 ºC	Typical fermentation temperature 8-15 ºC
Maximum growth temperature 37 ºC or higher	Maximum growth temperature 34 ºC
Does not ferment melibiose	Ferment melibiose
“Top” fermenter	“Bottom” fermenter

Despite *Saccharomyces* spp., many other yeast species are used as candidates for the production of specialty beer called non-Saccharomyces brewing yeasts. This innovation does not focus only on obtaining new products with more complex aromatic and flavor characteristics yet increasing the range of approaches to obtain different results in a growing market. Even though this particular field has just begun to be investigated, there is growing evidence of the wide potential for the application of non-Saccharomycesyeast in the brewing industry (Varela, n.d.). Several non-Saccharomyces yeast species are throughout spontaneous fermentation of certain beer styles (Belgium lambic beer and American coolship ales),

including *Meyerozyma guilliermondii, Debaryomyces spp., Pichiaspp., Wickerhamomyces anomalus, Brettanomyces anomalus* (Bokulich et al., 2012b; Spitaels et al., 2014).

Table 3 shows taxonomical status, different isolation sources (insect, soil, beer, cider, soft drink, and wine), and use of non-conventional yeasts for brewing (Basso et al., 2016).

Table 3 Taxonomical status, isolation sources, and use of non-conventional yeasts for brewing

Species	Anamorph [a]	Synonyms [a]	Isolation sources [b]	Application	Food safety [c]
Cyberlindnera saturnus		*Williopsis saturnus; Saccharomyces saturnus*	Insect, soil	Bio flavoring; low alcohol beer	Safe
Dekkera anomala	*Brettanomyces anomalus*	*Dekkera/Brettanomyces claussenii*	Beer, cider, soft drink	Bio flavoring; low calorie beer	Safe
Dekkera bruxellensis	*Brettanomyces bruxellensis*	*Brettanomyces lambicus; Brettanomyces custersii*	Beer, grape, wine, ginger ale	Bio flavoring; low calorie beer	Safe
Hanseniaspora uvarum	*Kloeckera apiculata*	*Kloeckeraspora uvarum; Hanseniaspora apiculata*	Grape must, fruits, soil, sour dough, insect, flowers	Bio flavoring	Safe
Pichia kluyveri		*Hansenula kluyveri*	Olives, fruits, flowers, soil	Bio flavoring	Safe
Torulaspora delbrueckii	*Candida colliculosa*	*Saccharomyces rosei; Saccharomyces delbrueckii*	Beer, fruits, soil, milk, moss, mushroom	Bio flavoring; low alcohol beer	Safe
Wickerhamomyces Candida colliculos		*Hansenula kluyveri*	Fruits; vegetables; molasses; jam; milk	Low alcohol beer	Safe
Wickerhamomyces anomalus	*Candida beverwijkiae*	*Pichia anomala; Saccharomyces anomalus; Hansenula anomala*	Beer, sour dough, fruits, sake, wine, oak	Bio flavoring; low calorie beer	Safe

3.2.3 Hop

The hop plant (*Humulus lupulus* L.) belongs to the family of the Cannabinaceae are known worldwide as an important raw material in beer production, mainly as an aroma and flavor agent, and also for increasing its foam and microbiological stability (Rodrigues Arruda et al., 2022). Hops were primarily used for their medicinal purposes, being associated with the treatments of both sleep and anxiety disorders, as well as an agent that activates gastric function as a bitter stomachic and enhances the performance of medicine formulations due to its antimicrobial and antifungal activities (Zanoli & Zavatti, 2008). α- and β-acids, also named humulones and lupulones, respectively, are found in hop lupulin glands (present in hop cones). They are also recognized as soft resins due to their solubility in hexane or yet hop bitter acids (Rodrigues Arruda et al., 2022). Hops have a pleasant and consistent bitterness dominant flavor attribute in beer. Hop-derived iso-α-acids are mainly responsible for beer bitterness and accurate determination of these primary flavor compounds are very important in relation to quality control (Jaskula et al., 2007).

Despite its popular application (i.e., brewing), hops can also be an interesting ingredient for other sectors of the food industry, such as food preservatives for meat and some processed

foods, and the production of flavoring constituents, especially for non-alcoholic beverages (Kramer et al., 2014a). Some studies concerning hop constituents as food preservatives proved the effect of α-acids, and β-acids, contained in commercial hop extracts, against foodborne bacteria and their potential application in marinated meat model systems (Kramer et al., 2014b). The bitterness found in Indian Pale Ale (IPA) beer is a notable trait that distinguishes it from other beer varieties. IPAs are recognized for their strong hop bitterness, which adds to their bold and occasionally robust flavor profile. This bitterness predominantly originates from the hops employed in the brewing process (Kawa-Rygielska et al., 2022).

3.2.4 Barley

Barley (*Hordeum vulgare* L.) is a versatile cereal that is widely used as human and animal feed, in brewing, and the production of ethanol for biodiesel. It is the fourth largest cereal crop following wheat, rice, and maize. In terms of nutrition, barley contains abundant starch, protein, dietary fiber, and minerals, along with antioxidant compounds, vitamins, and trace elements.(Panizo-Casado et al., 2020). It is an important source of food for a large population of cool and semi-arid areas of the world, where wheat and other cereals are less adapted. It contains starch which is converted to maltose and other sugars, and finally to alcohol and carbon dioxide. Other important functions of barley are color, flavor, and body which are fundamentally dependent on its roasting method (Singh et al., 2016).

Consequently, the whole grain of barley has a higher nutritional value and is commonly associated with more brownish colorations. Numerous health benefits of barley have been recognized worldwide by the European Food Standards Agency. This cereal is rich in (1-3) (1-4)-β-D-glucans which are one of the major soluble fibers in human nutrition. These compounds regulate blood glucose levels and reduce the effects of cholesterolemia and blood pressure, and therefore, reduce the risk of cardiovascular diseases. Moreover, they are recognized as sources of antioxidants, antibacterial, antitumor, and anti-inflammatory agents, and play a main role in the immunomodulation and regulation of the human gut flora (Bai et al., 2019). Furthermore, food with a high content of fiber increases intestinal transit, lowering the exposure to colonocyte carcinogens and increasing the satiation from a meal that can be useful in weight control (Panizo-Casado et al., 2020). Comparable to the rest of the grains, barley has significant amounts of phenolic compounds, especially in the external parts of the grain (cover, testa, and aleurone). The main compounds in the free phenolic fraction are flavonols, especially catechin,

procyanidins, and prodelphinidins, while phenolic acids, such as ferulic, coumaric, and vanillic acids, are major constituents of the bound phenolic fraction (Abdel-Aal & Choo, 2014).

Table 4 presemts the levels of major chemical compounds, total phenolic compounds (TPC), and antioxidant activity (AA) in six-row landrace barleys, categorized by spike density, aleurone layer color, and kernel size variation. (Panizo-Casado et al., 2020).

Table 4 The major chemical compounds, TPC, and AA

	Moisture (%)	Ash (%)	Starch (%)	Crude Fiber (%)	Protein (%)	Total phenols (mg GAE/100 g)	DPPH (mg TE/100 g)
Spike density							
Intermediate	14.6 ± 0.2^{b}	2.83 ± 0.04^{a}	53.5 ± 0.1^{a}	8.05 ± 0.1^{b}	11.7 ± 0.2^{a}	172 ± 11^{a}	0.82 ± 0.01^{a}
Lax	13.5 ± 1.1^{a}	2.79 ± 0.07^{a}	53.7 ± 0.9^{a}	7.43 ± 0.4^{a}	12.4 ± 0.8^{a}	184 ± 34^{a}	0.84 ± 0.09^{a}
Aleurone layer color							
White	13.4 ± 0.5^{bc}	2.79 ± 0.06^{a}	53.9 ± 1.0^{a}	7.32 ± 0.3^{a}	12.5 ± 0.4^{a}	188 ± 29^{a}	0.81 ± 0.06^{a}
Green	13.7 ± 1.2^{c}	2.79 ± 0.06^{a}	53.8 ± 0.9^{a}	7.49 ± 0.4^{a}	12.3 ± 0.8^{a}	184 ± 37^{a}	0.84 ± 0.09^{a}
Blue	13.2 ± 1.0^{ab}	2.77 ± 0.08^{a}	53.4 ± 0.9^{a}	7.35 ± 0.6^{a}	12.6 ± 0.8^{a}	180 ± 16^{a}	0.88 ± 0.05^{a}
Island							
Tenerife	13.6 ± 0.9^{b}	2.81 ± 0.05^{ab}	53.7 ± 0.9^{ab}	7.44 ± 0.4^{ab}	12.4 ± 0.8^{ab}	183 ± 34^{a}	0.86 ± 0.08^{bc}
La Palma	14.6 ± 1.1^{c}	2.77 ± 0.06^{a}	54.5 ± 0.3^{b}	7.71 ± 0.2^{bc}	11.6 ± 0.3^{a}	167 ± 47^{a}	0.94 ± 0.04^{c}
Gran Canaria	13.6 ± 1.3^{b}	2.77 ± 0.07^{a}	53.7 ± 0.9^{ab}	7.38 ± 0.4^{ab}	12.6 ± 0.7^{b}	186 ± 36^{a}	0.80 ± 0.09^{ab}
Lanzarote	12.4 ± 0.2^{a}	2.83 ± 0.06^{b}	53.5 ± 0.5^{a}	7.77 ± 0.2^{bc}	11.7 ± 0.3^{a}	186 ± 13^{a}	0.87 ± 0.06^{bc}
La Gomera	13.1 ± 0.8^{ab}	2.79 ± 0.09^{ab}	53.3 ± 1.1^{a}	7.29 ± 0.9^{a}	12.7 ± 0.7^{b}	182 ± 16^{a}	0.90 ± 0.05^{c}
Fuerteventura	12.9 ± 0.2^{ab}	2.84 ± 0.03^{b}	53.1 ± 0.4^{a}	7.99 ± 0.1^{c}	11.6 ± 0.2^{a}	193 ± 3.8^{a}	0.77 ± 0.01^{a}

3.2.5 Adjuncts

Adjuncts are non-malt substances used to provide extract in brewing. Corn, rice, unmalted barley, wheat starch, and sorghum are commonly used adjuncts in the brewing industry to supply fermentable carbohydrates. Brewers have utilized adjuncts for several reasons, including their ready availability in local markets, their ability to alter the sensory characteristics of beer, and notably, their cost-effectiveness compared to barley malt (Poreda et al., 2014b). The use of these alternative cereals for beer manufacturing is challenging in terms of the different physical and technological properties with new tastes from the original beer. The chemical composition of alternative grains is normally suboptimal to produce an alcoholic beverage such as traditional beer (Ceccaroni et al., 2018). Nevertheless, the use of adjuncts in brewing has certain drawbacks. For instance, incorporating unmalted cereals necessitates the utilization of a cereal cooker when applying infusion mashing (to enable starch gelatinization). Consequently, there is a need for investment in a cereal cooker setup. However, it is feasible to have the starch gelatinization accomplished in the mash tun, and then afterward, add the malt and water. Another disadvantage is that the raw materials' insufficient enzymatic power

prevents them from producing the best extract yield from the gelatinized grain. This drawback can be solved by using commercial enzymes. After all, these enzymes can reduce wort's foam potential, which has an impact on beer's foam potential. (Poreda et al., 2014a).

3.2.5.1 Wheat

Wheat (*Triticum aestivum* L.) is one of the major cereals in the world and is the main source of calories and protein (Caverzan et al., 2016). Wheat is a crucial cereal crop and holds the position of the second most significant grain globally, following corn, owing to its widespread production. Presently, wheat is the most extensively cultivated crop worldwide, and its trade across the world exceeds that of all other crops put together. This may be because wheat can adapt well to diverse latitudes and altitudes, growth temperatures, humidity levels, and soil types, making it agriculturally versatile (De Flaviis et al., 2022). Consequently, the health benefits of whole grains are particularly linked to the presence of antioxidants. Besides the most common antioxidants, such as vitamin C (tocopherols and tocotrienols), vitamin E, and carotenoids, wheat grains also contain some phyto-antioxidants, including phenolic acids and flavonoids (Yu et al., 2013). Phenolic acids, such as ferulic, syringic, p-coumaric, vanillic, and caffeic acids are natural antioxidants found in wheat grain, even though the largest amounts are in the insoluble bound form (Lv et al., 2012).

Table 5 presents the variation and heritability in bioactive components that are present in wholegrain fractions of wheat samples in the HEALTHGRAIN study (Shewry et al., 2013)

Table 5 Variation and heritability in bioactive components in wheat samples

	Mean content (µg/g dm) in HEALTHGRAIN diversity screen (150 lines)	Fold variation in HEALTHGRAIN diversity screen (150 lines)	Heritability (%) in HEALTHGRAIN diversity screen (150 lines)
Terpenoid			
Tocols	49.81	2.96	76
Sterols	843.8	1.39	57
Phenolic			
Total phenolic acids	657.42	3.60	28
Free phenolic acids	10.73	9.75	6
Conjugated phenolic acids	162.04	3.91	10
Bound phenolic acids	484.65	4.22	26
Alkylresorcinols	431.54	2.81	63
Methyl donors			
Betaine	1596	3.04	36

Choline	221	1.56	25
Trigonelline	3.10	16.13	59
B vitamins			
Folates B9	0.56	2.38	24

Wheat beer is a traditional light-colored top-fermenting beer that is brewed with at least 50% malted (e.g., German Weissbier) or unmalted (e.g., Belgian Witbier) wheat (*Triticum aestivum)* as an adjunct to barley (*Hordeum vulgare*) malt (Picariello et al., 2015). Since beer is generally produced from barley malt, wheat beer is considered a special type of beer. Its popularity has greatly varied over the recent years, due to the increase in demand for both industrial and craft brewing (Mastanjević et al., 2018). The aroma profile of wheat beer relies on many factors such as raw materials, and technical aspects in the brewery (size and geometry of the fermentation vessels, pressure conditions, aeration, etc.) that play a major role in the formation of aroma compounds along with the presence of different strains of *S. cerevisiae* provides enormous possibilities to create beers with different flavor attributes (Schneiderbanger et al., 2016). In contrast, wheat varies in its properties, brewers have not identified any specific wheat quality traits required for brewing purposes. Instead, wheat varieties are classified based on features like whether they are winter or spring varieties, their protein content, and the hardness or softness of the grain (Mascia et al., 2014). Beer brewed with a large wheat grist bill is expected to be largely unsuitable for a strict gluten-free diet due to an average protein content higher than all-barley-malt beers and the sequence specificity of toxic epitopes from wheat prolamins (Picariello et al., 2015).

3.2.5.2 Rice

Rice (*Oryza sativa* L.) is the staple food for about 3.5 billion people worldwide with a global annual production estimated at 720 million metric tons in 2012. Approximately 90% of the world's rice is grown and consumed in Asia, where about 60% of the world's population lives (Patindol et al., 2019.). Rice serves as a significant carbohydrate source, particularly in the form of starch composed of amylose and amylopectin. These starch components are predominantly found within the endosperm cells of mature brown rice, constituting around 90% of the dry weight of milled rice.(Amagliani et al., 2016). Moreover, the large amount of carbohydrates, rice includes a mild quantity of protein and fat as well as a source of vitamin B complexes like niacin, riboflavin, and thiamine along with minerals such as calcium (Ca),

magnesium (Mg), phosphorus (P) together with some traces amount of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Verma & Srivastav, 2020).

In addition to these constituents, some extra nutritional elements in small quantities are predominantly presented in different parts of rice (bran, germ fraction, and endosperm) that have performed the different biological activities known as bioactive compounds. These compounds are very valuable for human health, but they are not important for the growth and development of the human body (Verma & Srivastav, 2017).

Table 6 shows the chemical composition (amylose content, protein, fat, and ash) of rice starches from Brazil, China, India, Korea, and Nigeria. Regarding the chemical composition of rice starches, significant variances were discovered. The number of minor components determines how pure a starch is the lower the value, the purer the starch. These elements differ from quantification and starch isolation techniques. Starch's typical moisture, fat, and protein contents. (Kumar et al., 2021).

Table 6 Chemical composition of rice starch

Country	Samples	Amylose content %	Protein %	Fat %	Ash %
Korean	Rice variety "Boramchan"	22.22	0.33	0.90	0.17
China	Thailand mali rice (TML), Songnuo 2 (SN2; Waxy rice cultivars:), Simiao rice (SM; Indica rice cultivar), and Daohuaxiang rice (DH. Japonica rice cultivars)	1.5-38.62	0.08-0.39	0.06-0.08	-
India	Jhelum and Kohsar	4.9-6.3	0.40-0.52	0.25-0.33	0.16-0.33
Brazil	Rice cultivar "IRGA-424"	-	0.42	0.03	0.20
Nigeria	FARO 32, FARO 51, FARO 52, FARO 54, and NERICA	22.88-24.48	0.45-0.81	0.45-0.78	0.02-0.24

3.2.5.3 Maize

Maize or corn (*Zea mays* L.) is an important annual cereal crop belonging to the Poaceae family, which is among the top three crops worldwide, following rice and wheat. Maize grains are clustered in a diverse range of pigments, exhibiting a spectrum of colors from white and yellow to shades of purple, red, blue, and even black (Romero-Medina et al., 2020). Globally, maize is grown on 184 million hectares, with 1016 million tons produced annually (Dabija et al., 2021). In addition to its use as human food and animal feed, maize is a source for a huge

number of industrial products including maize grits, maize flakes, maize bran, and maize flour. Moreover, maize is used as a raw material for beer brewing, starch manufacturing, pharmaceutical starch, and bioethanol as a renewable source of energy and alternative to fossil fuels. (Malhotra, 2017).

Maize contains about 70% starch, other components being protein, fibers, fat, vitamins, and minerals. On top of that, maize involves some important phytochemical, bioactive chemical compounds naturally present in plants including carotenoids, phenolic compounds, and phytosterols, that provide human health benefits and the potential for reducing the risk of major chronic diseases (Rouf Shah et al., 2016).

Table 7 presents the variety of the chemical composition and nutritional value per 100g of edible portion of maize kernel. It includes N-p-coumaryl tryptamine, N-ferrulyl tryptamine, vitamin C, vitamin E, vitamin K, vitamin b1 (thiamine), b2 (niacin), b3 (riboflavin), b5 (pantothenic acid), b6 (pyridoxine), folic acid, and selenium. (Rouf Shah et al., 2016).

Table 7 Composition per 100g of maize kernel

Chemical Composition per 100g of maize Kernel	
Carbohydrates	71.88 g
Protein	8.84 g
Fat	4.57 g
Fiber	2.15 g
Ash	2.33 g
Moisture	10.23 g
Phosphorus	348 mg
Sodium	15.9 mg
Sulfur	114 mg
Riboflavin	0.10 mg
Amino acids	1.78 mg
Minerals	1.5 mg
Calcium	10 mg
Iron	2.3 mg
Potassium	286 mg
Thiamine	0.42 mg
Vitamin C	0.12 mg
Magnesium	139 mg
Copper	0.14 mg

Maize is now the most widely used local raw material and is extensively utilized as a brewing adjunct. Furthermore, the use of corn starch as an adjunct resulted in lower wort phenolic content compared to whole malt wort. Maize-adjunct worts have a lower amount of

total nitrogen compounds compared to all-malt worts, and the level of free amino nitrogen is almost twice as much in all-malt worts compared to adjunct worts (He et al., 2018). However, the addition of protease enhanced the amount of free nitrogen source in the wort, and the concentration of the free nitrogen source in corn wort (60%) was double that found in sorghum wort (30%) (Perez-Carrillo et al., 2012).

3.2.5.4 Grape

Grapes (*Vitis vinifera* L.) are among the world's largest fruit crops by production volume, ranking behind bananas, watermelons, and apples. In 2014, global grape production totaled approximately 75 million tons, with around 21 million tons dedicated to table grapes. (Aubert & Chalot, 2018a). Grapes are composed mainly of water, proteins, lipids, carbohydrates, vitamins, minerals, and compounds with important biological properties such as phenolic compounds (Karovičová et al., 2015). Additionally, grapes and products derived from the grape are rich in antioxidant compounds such as flavonoids, anthocyanins, tannins, and phenolic acids that provide nutritional benefits for consumer health (Dal Magro et al., 2016.). In particular, the beneficial impact of a moderate intake of wine and beer on cardiovascular disease has been linked to the polyphenol content contained in wine and beer (Veljovic et al., 2010).

Table 8 presents the Chemical composition, bioactive compounds, and volatiles of six table grape varieties. The SC ranged between 17.4 and 21.5 °Brix, with Muscat de Hambourg having the greatest SC and Italia Rubi having the lowest. In all varieties, glucose (75.8-93.9 g/L) and fructose (79.1-102.1 g/L) predominated, with sucrose (1.6-4.4 g/L) present at low concentrations. The levels of fructose were typically somewhat greater than those of glucose, except for Alphonse Lavallée (Aubert & Chalot, 2018b).

Table 8 Chemical composition and the volatiles of six table grape varieties

Compound	**White**			**Pink**	**Black**	
	Centennial Seedless	Chasselas	Italia	Italia Rubi	Alphonse Lavallee	Muscat de Hamburg
Soluble solid content	20.8 ab	17.6 bc	21.2 a	17.4 c	17.9 b	21.5 a
(Brix)	19.6–22.2	15.5–19.8	20.6–21.7	17.2–17.5	17.2–18.6	20.6–22.3
Titrable acidity (g/L	4.9 a	3.9 b	3.5 c	3.7 bc	3.8 bc	4.5 ab
tartaric acid)	4.8–5.1	3.6–4.1	3.3–3.6	3–4.5	3.3–4.4	3.9–5.9
Sugar						
Sucrose	3.7 a	2.8 ab	1.6 c	2.6 b	1.7 bc	4.4 a
	3.4–4.2	1.8–4.9	1.2–2	2.1–3	1.2–2.5	3.7–5.2
Glucose	88.6 b	77.3 bc	93.9 a	75.8 c	81.6 b	93.7 a
	85.1–92.3	67.7–87.7	93.3–95.6	73.5–78.1	78.5–84.5	84.9–103.4
Fructose	102.1 a	80.9 bc	100.3 a	79.7 c	79.1 c	95.9 ab

	100.2–103.5	70–92.4	99.1–101.2	77.6–81.5	76–82.	88.5–104.5
Total	194.4 a	161.0 b	195.9 a	158.1 b	162.3 b	194.1 a
	188.7–199.7	140.0–182.9	194.6–198	153.9–162.4	157.0–168.2	178.6–211.6
Total sugar/TA	39.7 b	42.2 b	56.3 a	44 b	43.3 b	43.3 b
	37.6–41.7	34.3–56.5	54.9–59.2	34.5–53.6	35.6–50.9	35.1–45.9
Organic acids (g/L)						
Tartaric acid	6.2 a	5 bc	5.7 ab	5.6 b	4.3 c	4.9 c
	6.0–6.4	4.3–5.7	5.2–6.2	5.1–5.9	4.0–4.8	4.7–5.1
Malic acid	1.7 b	1.5 c	1.9 b	1.8 b	2.2 b	2.9 a
	0.8–2.6	1.3–1.6	1.8–2.0	1.7–1.9	1.2–3.3	2.4–3.5
Total	7.9 a	6.5 b	7.6 ab	7.3 ab	6.5 ab	7.8 a
	6.9–8.9	5.7–7.2	7.0–8.2	7.3 ab	5.2–8.1	7.4–8.3

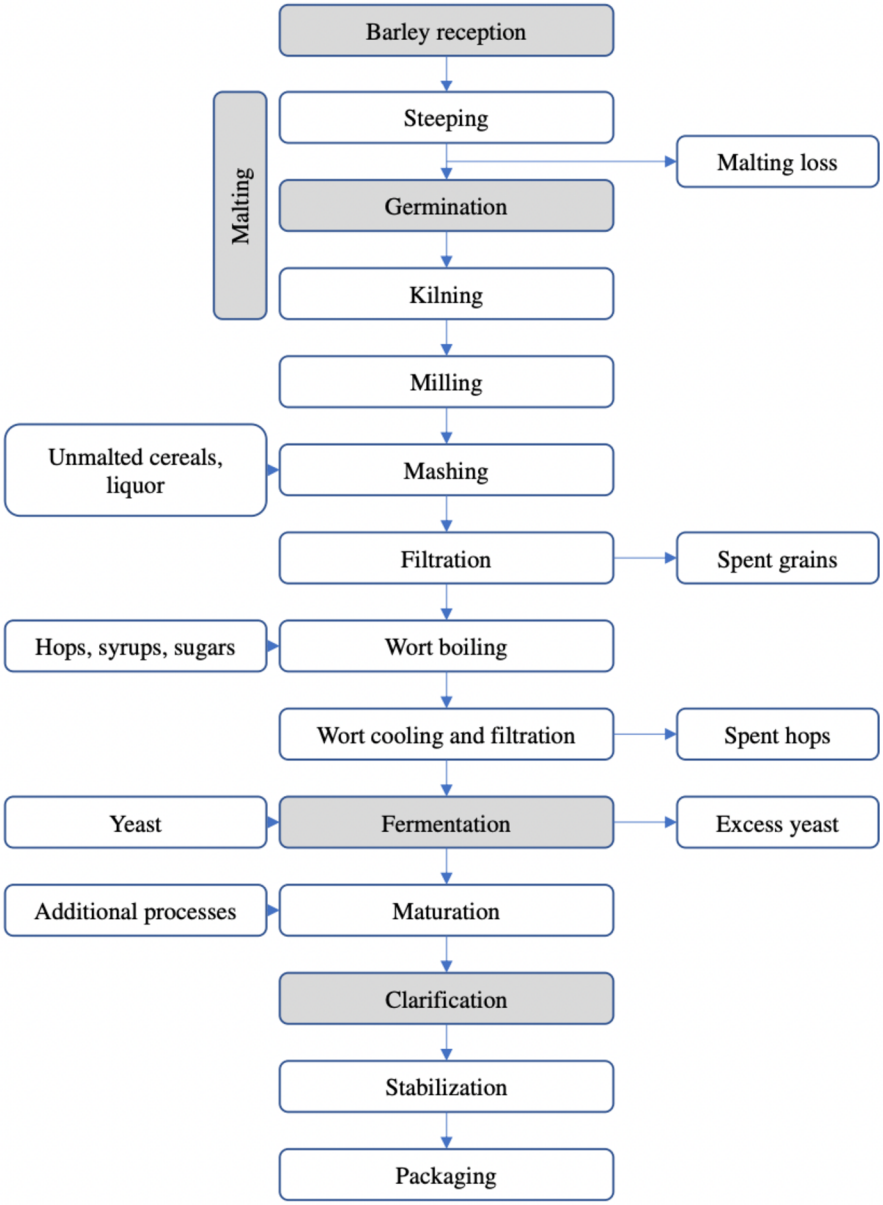
Consequently, special beers made with fruit adjuncts have a complex sensory profile marked by well-balanced and harmonious fruity flavors. One of the most appealing products produced by micro and craft breweries is fruit beer, which also boasts some intriguing qualities like a high content of polyphenols (mostly phenolic acids), fragrance compounds, and a strong antioxidant potential (Siesto et al., 2023b). As expected according to the grape composition, most beers produced with fruit adjuncts generally contain a higher concentration of polyphenols, increased antioxidant activity, better aroma, a higher content of volatile compounds as well as an improved sensory profile (Gasiński et al., 2022b; M Paiva et al., 2021). In 2015, Italian Grape Ale (IGA) beers, a new fruit-style beer was included as a new provisional subcategory of special-type fruit beers, which is a product resulting from the marriage between beer and wine. The brewing process is carried out in the presence of determining quantities of grape must (Castro Marin et al., 2021). Many small regional breweries are currently employing this innovative brewing method, which is anticipated to better express the relationship with the region by integrating the diversity of Italian grape varieties with the brewer's invention. IGA beers are brewed with pilsener/pils or other pale base malts with grapes or grapes must added at different phases of fermentation, ranging from 5% to 40% of the total wort composition (boiling, primary, or secondary fermentation or bottling) (Siesto et al., 2023).

3.3 The Beer Brewing Process

The beer brewing process involves several sections separated into two parts: brewing and aging. The brewing part generally operates at higher temperatures while the following aging part operates at lower temperatures. Consequently, the milled malt is mixed with hot water in the mash tun which results in a sugar-rich liquid, called wort. This wort is then transferred into the lauter tun where it is separated from the grains. During the next step, called wort kettle, the wort is boiled with hops. This step is very important as it affects the flavor, colour, and aroma of the brewed beer. Afterward, the boiled wort is transferred into a whirlpool where solid

particles are removed to allow fermentation, where the aging part of the beer brewing process begins (Faruk Pasic, 2019). The goal of brewhouse operations is to extract malt or adjunct sugars with maximum efficiency. A few chemical transformation processes occur, that is, the oxidation of polyphenols, the formation of lipid-protein complexes, and the precipitation of proteins throughout the brewing process. The nitrogen and carbohydrate composition of the wort depends on the enzyme-to-substrate ratio, that is, the ratios of a-and b-amylases/starch and endopeptidases/proteins. These ratios can be adjusted by the following techniques: the use of substrates without enzyme activity (adjuncts) reduces the amount of nitrogen in the wort, thermal destruction of enzymes by boiling during decoction mashing, adjusting the malt/water ratio, adjusting the pH to influence the activity of the enzymes, which has an influence on the activity of certain enzymes during mashing (Wiley Jonh, 2007).

Figure 2 shows a schematic overview a of the brewing process and steps, where the input flows, output flows, and possible decontamination strategies could be applied (blocks in grey) are also indicated (Pascari et al., 2018).



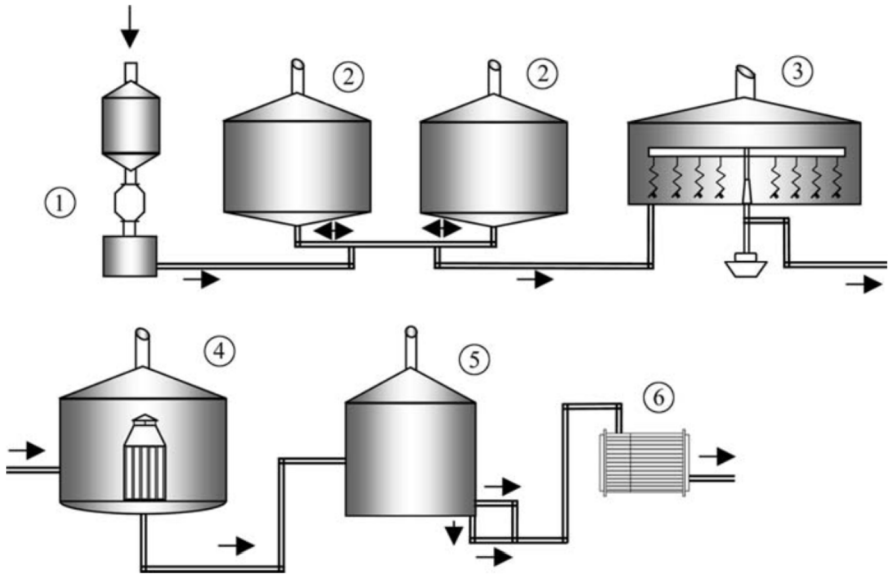
(Pascari et al., 2018).

Figure 2 *Beer production scheme and steps where possible decontamination strategies could be applied (blocks in grey)*

The technology of brewhouse (wort production) can be organized into six-unit processes: grinding of malt and adjuncts, mashing, wort filtration, wort boiling, wort clarification, and wort cooling and aeration. In a classical brewery, the malt is milled, and the grist is mixed with brewing water (“mashing in”). Mashing can be accomplished using the infusion method or the decoction method. An adjunct cooker is necessary in the case of using adjuncts with a high gelatination temperature. In the filter vessel or the mash filter, the wort ("the liquid extract") is separated from the insoluble material called the spent grain. Next, the wort is boiled in a boiling

vessel. After boiling, the hot trub is removed by a whirlpool, a sedimentation tank, or a centrifuge, and cooled using a plate heat exchanger until fermentation temperature and aerated (Wiley Jonh, 2007).

Figure 3 shows schematically the different unit operations in the brewhouse. Wort production in a classical brewhouse: 1. malt grinding, 2. mash vessels (decoction mashing), 3. wort filtration in a filter vessel, 4. wort boiling, 5. wort clarification in a Whirlpool, 6. wort cooling (heat exchanger) (Wiley Jonh, 2007).



(Wiley Jonh, 2007)

Figure 3 *Wort production in a classical brewhouse*

3.4 Malting

A controlled germination process to produce malt is called malting. The malting process's purpose is to transform insoluble starch and proteins of barley grains into a substrate capable of dissolution and extraction by hot water. Barley is the primary cereal used in malt production, which contains high levels of β-glucans and phenolic compounds (Sharma & Gujral, 2010). There are three stages of the malting process: steeping, germination, and kilning. Steeping is a process started under specific humidity and conditions of temperature (controlled cycles of water spraying or immersion and aeration until grain water content reaches 42–48%), along with higher concentrations of reducing sugars and amino acids. The humidity of the barley after steeping is influenced by the type of malt that is projected to be obtained (42–44% for Pilsner and 47–48% for dark beers). Steeping's purpose is to activate the enzymes involved in germination and generate favorable humidity conditions inside the grain. It generally comes

at about 10–15 °C where, after about 30–50 h, the water enters the kernel and the first signs of germination appear (Pascari et al., 2018).

Germination allows the activation of all enzymatic equipment for the break of reserves of proteins and starch and hydrolysis of cell walls (Carvalho et al., 2016a). This process starts a few hours after water penetration into the grain during steeping and begins with the transport of gibberellic acid (growth promoter) to the aleurone layer where enzyme production and activation take place. The following enzymes are synthesized: amylases and dextrinases, cytolytic enzymes, proteolytic enzymes, lipases, lipoxygenases, and phosphatases. (Oliveira et al., 2012). The germination process is regulated by controlling the growth of rootlets that are expected to grow to a length of between 1.5 and 2 times the original length of the grain (Carvalho et al., 2016a).

The kilning and roasting prepare the malt for storage and transportation if needed. It normally takes place at several temperature scales: < 50 °C until the water humidity of grains reaches 10–12% and then the temperature is gradually increased to 80–90 °C. The temperature is chosen aiming to reduce at a minimal level the degradation of the enzymes. The thermal processing steps have a huge impact on colour and flavor of malt, depending on the time course, temperature, and moisture content (Pascari et al., 2018). The steps aim to reduce the moisture content of green malt and to a condition that ensures stability during transportation and storage (approximately 5%) (Carvalho et al., 2016a).

3.5 Milling

The main purpose of milling the malt and other grain is to increase the contact surface between the brewing liquor and malt. Generally, hammer and roller mills are used to obtain the optimum results because in this way the husks that barriers the extraction of tannins and other undesirable compounds are almost intact. Finer particles provide a better breakdown of malt into fermentable materials such as sugars and assimilable nitrogen compounds while mashing. Nevertheless, too small particle size may have a negative effect by decreasing filtration yields and increasing wort turbidity. (Pascari et al., 2018). Some studies have found that the capability of milling is not only expressed in the size of final granules however it should be evaluated together with mashing temperature levels because the activity of α-amylase depends on both granules size and treatment temperature (Mousia et al., 2004).

3.6 Mashing

Mashing is the process of mixing milled malt and a large amount of water (approximately 17 kg of malt are needed for 1 hL of beer) under specific temperatures to allow the conversion of starches into fermentable sugars and to activate all the enzymatic equipment present (inactivated during kilning). There are primarily two types of enzymes: those that act on sugars and those that act on proteins. The physical conditions applied while mashing aim to optimize the efficiency of the enzymes according to their different optimal temperatures. There are four temperature scales which are normally held for some time to allow the following changes:

- 45 to 50 °C for β-glucans and protein hydrolyzation,
- 62 to 65 °C for maltose production,
- 70 to 75 °C for saccharification, a
- 75 to 78 °C for α-amylases activation and finishing of mashing. (Pascari et al., 2018).

An alternative mashing process is called "decoction mashing", where different temperatures are performed by removing repetitively a part of the mash, boiling it, and mixing it back. During this step, it is crucial to control all possible parameters such as temperature and heating time and continue with pH (optimal being pH = 5.2), oxygenation level, and stirring speed (Pires & Brányik, 2015).

3.7 Wort Separation and Boiling

The aim of wort boiling is the evaporation of water and unwanted volatile compounds, isomerization of humulones, as well as fixation of wort composition by inactivation of enzymes, removal of proteins, and sterilization of wort. Nevertheless, although the boiling stage can positively contribute to the formation of color during beer storage, it should be carefully monitored, as it may lead to the formation of a non-biological haze by the oxidation of polyphenols derived from malt and hop vegetative matter (Kandylis et al., 2022). This process is performed after the separation of the solid particles, and hops are added at this step. The wort can also be improved by adding sugars, syrups as well as a seasoning for instant coriander seeds, orange peel, etc (E. Pires & Brányik, 2015).

Hop (*Humulus lupulus*) is used in the brewing process during the boiling phase to improve the quality and stability of beer and to introduce the bitter taste and characteristic hoppy aromas, which leads to a desirable flavor for consumers. Hop acids, hop oils, and

polyphenols are the most important biochemical markers that differentiate the hop varieties. The hop acid includes α-acids (humulone, colupulone, and adhumulone) and β-acids (lupulone, colupulone, and adlupulone) (Kandylis et al., 2022). The hop boiling step typically lasts for 45-60 min or longer. It varies as a function of the boiling time, hops used, hoping rating, and the moment the hops are introduced (in the beginning, in the middle, and at the end of the process). The key processes taking place during wort boiling are:

- enzyme inactivation,
- evaporation of water and volatile compounds,
- protein precipitation,
- sterilization,
- isomerization of hop α-acids,
- Maillard reactions, and thus flavor modulation (Pascari et al., 2018).

The α-acids are tasteless; however, they are isomerized to the bitter-tasting iso-α-acids or isohumulones upon wort boiling. During boiling, approximately half of the α-acids undergo isomerization, resulting in less than a quarter of their original bittering potential being retained in the beer. This happens because of the restricted solubility of the α-acids in beer and the slightly acid wort (pH 5–5.5) (Caballero et al., 2012). On the other hand, the volatile fraction present in the hop oil (0.5–3%in hops), along with the non-volatile fraction contained in the hop polyphenols (3–6%) contributes to a full mouthfeel sensation during the beer tasting. To stabilize the foam head, the processed hop advanced products, such as reduced iso-α-acids, improve foam stability to a greater extent than iso-α-acids (Sturm et al., 2020).

3.8 Fermentation and Maturation

Fermentation is the process in which fermentable carbohydrates are transformed into alcohol, carbon dioxide, and a range of secondary compounds such as esters, higher alcohols, and volatile compounds by the yeast Saccharomyces genus. An effective brewing fermentation is required to ensure a high-quality product, as most of the flavour-active compounds of the beer are produced at this stage of the process (Kandylis et al., 2022). Types of beer refer to the use of different yeast strains. As reported in section 3.1, there are two most common technologies known: ale or top fermentation using *Saccharomyces cerevisiae*, and lager or bottom fermentation using *Saccharomyces pastorianus*. The metabolic activity of yeasts is possible at a temperature range of 2 to 30 °C and the initial yeast concentration at inoculation must be 107 cells/mL. Normally, the fermentation temperature is 7–15 °C for lager beers and 18–25 °C for ale beers during 7–9 days (Pascari et al., 2018). However, all strains of

Saccharomyces produce ethanol as an end-product of fermentation, the production of the major aroma-active compounds is strictly dependent on the yeast strain chosen for the fermentation and has a big impact on the beer flavor (Olaniran et al., 2017). The most important elements produced by yeast, which will determine the final quality of the product, are vicinal diketones (SDKs), higher alcohol, and esters. Higher alcohols and esters can be considered pleasant and desirable volatile constituents in beer, depending on their concentration level, while SDKs are frequently considered off-flavors (E. J. Pires et al., 2014). During fermentation, oxygen plays an important role as it is required by all yeast cells to support the synthesis of sterols and unsaturated fatty acid components of the cell membranes. It is also required for lipid synthesis, which is necessary to maintain the integrity and function of the plasma membrane as well as cell replication (B. R. Gibson et al., 2007). On the other hand, an excess of oxygen may damage cell components, contribute to cellular aging, and finally lead to cell death. Therefore, to obtain a high-quality product, it is necessary to achieve optimum oxygen levels (B. R. Gibson et al., 2008).

The primary purpose of maturation (also known as secondary fermentation) is to improve and stabilize the beer taste after fermentation (CO_2 elimination and removal of some undesirable volatile compounds) (Rodman & Gerogiorgis, 2016). During this step, other processes are carried out such as beer clarification, yeast sedimentation, and flavor formation of the final product. The process of maturation normally lasts from 1 to 3 months and requires lowering of the temperature (cold break) to around 0 °C. Secondary fermentation is usually practiced (2 million cells/mL) and the addition of priming sugars is acceptable. During the conditioning stage, proteins and tannins combine and form larger molecules, leading to the sedimentation of these high-mass molecules and resulting in beer clarification. Proteins could also be eliminated through the addition of enzymes, the presence of additional tannins, or by adsorption onto the surface (nylon membranes, silica gels, etc.) (Pascari et al., 2018). The clarification process can be quickened by filtration or centrifugation at low temperatures, 0 to −1 °C. Beer volume is then filtrated to remove the yeasts and achieve a clear final product. The product is transferred to aging tanks for more prolonged storage (Rodman & Gerogiorgis, 2016).

3.9 Effect of Operating Conditions on Beer Quality

3.9.1 Influence of Malt Composition

In beer production, malt is produced from barley grains in a process called malting which is used as a source of starch, contributing to beer's color and organoleptic characteristics. In addition, it also plays an important role in the oxidative stability of beer and is a natural source of antioxidants that can limit reactions caused by reactive oxidizing species (ROS) (Carvalho et al., 2016b). The antioxidants found in beer primarily come from malts, which are categorized as colored, caramelized, or roasted based on the malting process.(Petrucci et al., 2021). During the malting process, the extractability of these compounds is increasing mostly due to enzymatic processes and better friability (Carvalho et al., 2016b).

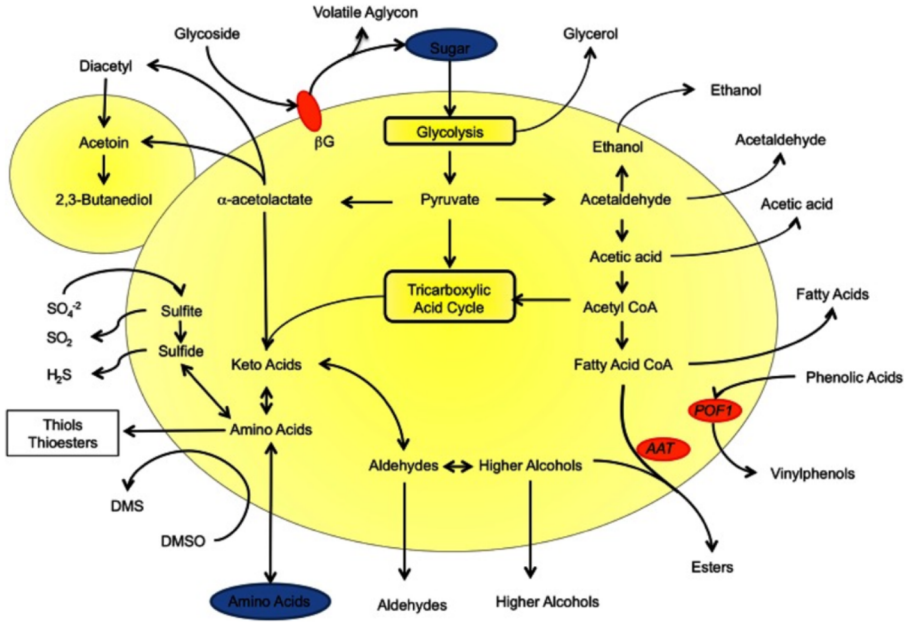
The technical steps used in brewing and malting have a substantial impact on the composition of malts and the resulting beer. These processes affect not only the extract, alcohol, and protein content of the final product but also influence the presence of bioactive components such as antioxidants. According to the study (Schwarz et al., 2012) focused on how mashing-in temperature affects the release of polyphenols. They found that the optimum temperature for phenolic acid release from the malt is between 40–45°C, while at temperatures above 65°C no enzyme activity related to the release of phenolic acids was detected. Authors (Fumi et al., 2011) investigated the polyphenols in all-malt worts and maize adjunct worts, and their fate during the main brewing steps. They reported higher phenolic content in all-malt worts than in maize adjunct worts, moreover, they noted that the overall brewing process reduces by 50% the initial content of total phenols. Other authors (Zhao, 2015) studied the influence of processing stages on the profile of phenolic compounds from barley to the final beer product. It was found that during malting and mashing, their amount had significantly increased but decreased remarkedly during the subsequent fermentation and storage.

3.9.2 Influence of Microorganisms

The production of quality beer relies on the activity of fermenting yeasts that are qualified for good fermentation yield-efficiency, affecting the aroma and flavor of the beverage (Marongiu et al., 2015). Brewing yeast strains can use numerous carbohydrates (glucose, sucrose, fructose, maltose, galactose, raffinose, and maltotriose), and the special characteristic differentiating ale and lager yeasts is the ability of lager yeasts to ferment melibiose (Capece et al., 2018). The aroma profiles of beer are generally attributed to biochemical activities during fermentation in the yeast cell, in which the carbohydrates in the must are converted into ethanol

and volatile compounds (such as alcohols and higher esters), which are intermediates and secondary products of yeast metabolism. These volatile compounds are different from the aromatic compounds that originate in malt and hops and have a significant impact on the aroma and taste of beer (Capece et al., 2018). Fermentation conditions are important for bacteria to grow, and contamination can delay or extend fermentation which causes various flavours and odours. Specific gravity, pH, and flavor are normally checked during preparation, and microbiological analysis is carried out only if problems occur during fermentation (Sclifos, 2022).

Figure 4 presents a simplified outline that encapsulates the primary metabolic pathways of *Saccharomyces*, which play a pivotal role in shaping the quality of beer and influencing its flavor profile. (Sclifos, 2022).



(Sclifos, 2022)

Figure 4 *Metabolic activity of Saccharomyces that influences the beer's quality*

4 Materials and Methods

4.1 Beer Production

In this study, the whole experiment was carried out in the food technology laboratory of the Department of Agriculture, Food, and Environment (DAFE) of the University of Pisa (UNIPI). We performed different experimental trials, using an experimental microbrewery plant. Five different raw materials such as red grape, white grape, orange skin, honey, and sugar were used as the main raw material in this experiment. The first experimental test of beer production was the creation of an APA-style beer, working with volumes equal to half of the plant's capacity.

STEP 1: We lightly ground the malt in a roller mill.

STEP 2: We filled the pots for the mash and the sparge with the volumes of water calculated according to the recipe created: 27.9 liters for the mash and 26.72 liters for the sparge. For water with a pH that is too high, a correction with lactic acid is applied in order to reach a pH of about 3.4.

STEP 3: We light the burners placed under the pots until a water temperature of 60°C is reached.

STEP 4: Once the ideal water temperature has been reached (60°C), we poured 9.3 kg of ground malt for the mash-in phase for 60 minutes.

STEP 5: After 60 minutes, we brought the water to 78 ° C for 15 minutes, thus practicing the mash-out phase.

STEP 6: We carried out an iodine test on mashed water to verify that the sugars had been extracted.

STEP 7: We carried out the first filtration of the must by filling a jug from the bottom tap and pouring the contents from the top of the pot several times until we obtained a very filtered must.

STEP 8: We mounted the filter for the sparge and practiced the sparging until we reached the desired volume of 46 liters in the boiling pot.

STEP 9: we checked the initial pre-boiling density with a densimeter.

STEP 10: Start boiling lasting 60 minutes.

STEP 11: Insertion of 20 grams of cascade hops 60 minutes from the end of boiling.

STEP 12: Insertion of 20 grams of cascade hops 30 minutes from the end of boiling.

STEP 13: Insertion of 40 grams of cascade hops 5 minutes from the end of boiling.

STEP 14: We let it cool for a few minutes and whirled it with a flat ladle to settle the solid residues in the center.

STEP 15: With a heat exchanger we transferred the wort from the boiling pot to the fermenter, at a temperature of 20 °C.

STEP 16: We added two 10 grams of Mangrove Jack's M54 yeast sachets.

STEP 17: we closed the fermenter and affixed the bubbler with water.

STEP 18: After a month we made refermentation in the bottle by applying a priming with four different of sugars to obtain a final concentration of hexoses of 3 g/L:

- Sucrose;
- Honey;
- Red grape must;
- White grape must.

For 4 bottles added with sucrose, we made an infusion of citrus peels (5% w/v) in the must.

4.2 Chemical Analysis

4.2.1 pH and Total Acidity

To eliminate excess carbon dioxide, 200 mL of beer was agitated in a 500 mL flask at a temperature of 20°C. The beer was then filtered through a dry folded filter paper in a funnel into a second conical flask. This agitation and filtration process were repeated to ensure complete degassing of the beer. Subsequently, the pH of the beer was determined using a precalibrated pH meter.

The determination of the total acidity in the beer samples was measured through potentiometric titration. A solution was prepared by adding 10 mL of beer to 50 mL of distilled water, and the resulting mixture underwent titration with 0.05 N sodium hydroxide. The volumme of NaOH needed to achieve a pH of 8.2 was established through the utilization of a pH meter. Total acidity was quantified in units of tartaric acid equivalents.

4.2.2 Beer Bitterness

10 ml of lager beer, 1 mL of HCl at 3 N concentration and 20 mL of isoctane with 50 μL of octanol are placed in a 50 mL test tube to prevent the formation of foam which must be added under the chemical hood using the aid of a propipetta. Then shake the test tube for about ten minutes and let it stratify for the time necessary so that the aqueous layer can be separated from the organic layer. To facilitate the extraction of the organic phase, it is possible to perform centrifugation with subsequent separation of the supernatant for 3 minutes at 3000 rpm. With this method, 3 phases can be formed consisting of beer on the bottom, a protein emulsion in the

middle, and a transparent phase on the upper part constituting the organic phase necessary to carry out the reading on the spectrophotometer. Then the supernatant was recovered and read on the spectrophotometer at a wavelength of 275 nm. The results were expressed as EBU. The average values of two determinations were used for data analysis.

The calculation was made with the formula:

$$\text{Bitterness (EBU)} = A_{275nm} \text{ x } 50$$

where A_{275nm} is the absorbance at 275 nm measured against pure iso-octane as a reference.

4.2.3 Color

The beer's absorbance was measured at a wavelength of 430 nm using a 10 mm cuvette. The colour in EBC (European Brewing Convention) units was determined by multiplying the absorbance by a specific factor. The beer samples underwent degassing through gentle stirring with a low-speed magnetic stirrer and the sample was filtered through a 0.45 μm membrane filter.

The color of the undiluted sample was calculated using the formula:

$$\text{Color (EBC)} = \text{A x f x } 25$$

where A is the absorbance at 430 nm in a 10mm cuvette and f is the dilution factor (12).

The color of the beer samples was evaluated also using a tristimulus colorimeter (Eoptis, Mod. CLM-196 Benchtop, Trento, Italy). The color was determined based on the chromatic coordinates including lightness (L*), green-red (a*), and blue-yellow (b*) components of the CIE L*a*b* color system. Additionally, the Chroma value (C*), indicating color saturation, and the hue value (H*), representing tonality, were calculated using specific relationships (Taglieri et al., 2021):

$$C* = \sqrt{a*^2 + b*^2} \quad (1)$$

$$H* = tan^{-1}(b*/a*) \quad (2)$$

The color difference between samples was expressed as ΔE^*_{ab}:

$$\Delta E * ab = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b *^2} \quad (3)$$

ΔE^*_{ab} quantifies the difference between two colors., providing a standard measurement for comparing the perceived color difference between a reference color and a sample color. The lower the ΔE^*_{ab} value, the less distinguishable the color difference is to the human eye.

4.2.4 Alcohol Content

The alcoholic degree is a mandatory indication on the labeling of beverages that contain more than 1.2% alcohol by volume (wine, beer, liqueurs, spirits, etc.). It must be indicated in the visual field of the label where the name of the drink and the net quantity appear.

The method is developed in two phases:

1. Separation of alcohol from beer by distillation

The beer sample to be analyzed is subjected to magnetic stirring for 24 hours to eliminate the CO_2 present and then filtered on a pleated filter to eliminate any suspended particles. A steam current distillation is then carried out to extract only the alcohol from the matrix. $Ca(OH)_2$ is added to the solution to block the more volatile acid compounds, while antifoam is added to avoid that during the distillation when the liquid reaches the distillation column. The steam stream distiller is also equipped with an automatic system that allows the process to be stopped when the desired quantity of distillate by weight is reached.

2. Measurement of the density of the hydroalcoholic solution obtained

The solution of water and alcohol obtained is subjected to density measurement to trace the quantity of alcohol present. This measurement is performed with a hydrostatic balance which will directly give the alcoholic content expressed as % v/v. The measurement is normalized to the calibration temperature of the instrument (20 °C) with the values shown in the table by the manufacturer.

4.2.5 Acetic Acid

The laboratory protocol for acetic acid follows Megazyme Company's manual assay procedure.

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: 25 ^{0}c

Sample solution: 0.3 to 25 µg of acetic acid per cuvette (in 0.1-2.0 mL sample valume)

Read againt air or againt water (without a cuvette in the light path).

Table 9 Procedure for acetic acid mesurement

Pipette into cuvettes	Blank	Sample
distilled water (at 25 ^{0}c)	2.10 mL	2.00 mL
sample	-	0.10 mL
solution 1 (buffer)	0.30 mL	0.30 mL
solution 2 (NADH/ATP/PEP/PVP buffer)	0.20 mL	0.20 mL
solution 3 (CoA)	0.02 mL	0.02 mL
solution 4 (D-LDH/PTA/PK)	0.02 mL	0.02 mL
Mix, read the absorbences of the solutions (A_1) after aprox. 2 min and start the reaction imidiately by addition of:		
suspension 5 (AK)	0.02 mL	0.02 mL
Mix and read the absorbances of the solution (A_2) at the end of the reaction (aprox. 4 min).		

Calculation: Determine the absorbance difference (A_1-A_2) for both blank and sample. Subtract the absorbance difference of the blank and the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{acetic acid}}$. The values $\Delta A_{\text{acetic acid}}$ should be at least 0.100 absorbance units to receive sufficiently accurate results.

The concentration of acetic acid can be calculated using the following equation:

$$c = \frac{V \times MW}{\varepsilon \times d \times V} \times \Delta A\text{acetic acid} \qquad [g/L]$$

where:

V = final valume [mL]

MW = molecular weight of acetic acid [g/mol]

4.2.6 D-glucose and D-Fructose

The laboratory protocol for acetic acid conforms to Megazyme Company's manual assay procedure.

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: 25 ^{0}C

Final valume: 2.32 mL (D-glucose); 2.34 mL (D-fructose)

Sample solution: 4-80 μg of D-glucose plus D-fructose per cuvette (in 0.10-2.00 mL sample valume)

Read againt air or againt water (without a cuvette in the light path).

Table 10 Procedure for D-glucose and D-fructose measurement

Pipette into cuvettes	Blank	Sample
distilled water (at 25 ^{0}c)	2.10 mL	2.00 mL
sample	-	0.10 mL
solution 1 (buffer)	0.10 mL	0.10 mL
solution 2 ($NADP^+$/ATP)	0.10 mL	0.10 mL
Mix the solutions, measure the absorbances (A1) after approximately 3 minutes, and initiate the reactions by adding:		
suspension 3 (HK/G6P-DH)	0.02 mL	0.02 mL
Mix and read the absorbances of the solution (A2) at the end of the reaction (aprox. 5 min). If the reaction has not stopped after 5 min continue to read the absorbancees at 2 min intervals until the absorbances remain the same over 2 min. Then add:		
suspension 4 (PGI)	0.02 mL	0.02 mL
Mix and read the absorbances of (A3) at the end of the reactions (aprox. 8-10 min)		

Calculation: Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$.

Determine the absorbance difference (A_3-A_2) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-fructose}}$.

Typically, the differences in values of $\Delta A_{D\text{-glucose}}$ and $\Delta A_{D\text{-fructose}}$ should as a rule be at least 0.100 absorbance units to to ensure sufficiently accurate results.

The concentration of $\Delta A_{D\text{-glucose}}$ and $\Delta A_{D\text{-fructose}}$ can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times V} \times \Delta A \qquad [g/L]$$

where:

V = final valume [mL]

MW = molecular weight of $\Delta A_{D\text{-glucose}}$ and $\Delta A_{D\text{-fructose}}$ [g/mol]

ε = absorbance coefficient at 340 nm of NADPH

= 6300 [1 × mol^{-1} × cm^{-1}]

d = light path [cm]

v = sample volume [mL]

It follows for D-glucose:

$$c = \frac{2.32 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{D-glucose}} \quad [\text{g/L}]$$

$$= 0.6634 \times \Delta A_{\text{D-glucose}} \quad [\text{g/L}]$$

for D-fructose:

$$c = \frac{2.34 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{D-fructose}} \quad [\text{g/L}]$$

$$= 0.6692 \times \Delta A_{\text{D-fructose}} \quad [\text{g/L}]$$

If the sample was diluted during preparation, the result must be multiplied by the dilution factor, denoted as F.

4.2.7 Total Lactic Acid

The laboratory protocol for acetic acid conforms to Megazyme Company's manual assay procedure.

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: 25 ^{0}C

Final valume: 2.24 mL (D-lactic acid); 2.26 mL (L-lactic acid)

Sample solution: 0.5-30 µg of total lactic acid per cuvette (in 0.1-1.5 mL sample valume)

Read againt air or againt water (without a cuvette in the light path).

Table 11 Procedure for total lactic acid measurement

Pipette into cuvettes	Blank	Sample
distilled water (25°C)	1.60 mL	1.50 mL
sample	-	0.10 mL
solution 1 (buffer)	0.5 mL	0.50 mL
solution 2 (NAD^+)	0.10 mL	0.10 mL
suspension 3 (D-GPT)	0.02 mL	0.02 mL
Mix, read the absorbances of the solutions (A_1) after (aprox. 3 min), and start the reactions by adding:		
suspension 5 (D-LDH)	0.02 mL	0.02 mL
Mix, read the absorbances of the solutions (A_2) at the end of the reaction (aprox. 5 min). If the reaction is still ongoing after 5 min, continue monitoring the absorbances at 1 min intervals until the absorbances either either stabilize or show a consistent increase over 5 minutes.		
suspension 4 (L-LDH)	0.02 mL	0.02 mL
Mix, read the absorbances of the solutions (A_3) at the end of the reaction (aprox. 10 min). if the reaction has stopped after 10 min, continue to read the absorbances at 5 min intervals until the absorbances either remain the same or increase constantly over 5 min.		

If this creep rate is greater for the sample than blank, extrapolate the absorbances (sample and blank) back to the time of addition of suspension 4 or 5.

Calculation:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{D-lactic acid}}$.

Determine the absorbance difference (A_3-A_2) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{L-lactic acid}}$.

The concentration of D- and L-lactic acid can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times V} \times \Delta A_{\text{D-lactic acid}} \quad [\text{g/L}]$$

where:

V = final valume [mL]

MW = is the molegular weight of lactic acid [g/mol]

ε = exstinction coefficient of NADH at 340 nm

= 6300 [1 × mol^{-1} × cm^{-1}]

d = light path [cm]

v = sample valume [mL]

It follows for D-lactic acid:

$$c = \frac{2.24 \times 91.1}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{D-lactic acid}} \quad [\text{g/L}]$$

$$= 0.3204 \times \Delta A_{\text{D-lactic acid}} \quad [\text{g/L}]$$

for L-lactic acid:

$$c = \frac{2.26 \times 180.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{L-lactic acid}} \quad [\text{g/L}]$$

$$= 0.3232 \times \Delta A_{\text{D-fructose}} \quad [\text{g/L}]$$

If the sample was diluted during preparation, the result must be multiplied by the dilution factor, denoted as F.

4.2.8 Glycerol

The laboratory protocol for acetic acid conforms to Megazyme Company's manual assay procedure.

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: 25°C

Final valume: 2.34 mL

Sample solution: 0.8-35.0 µg of glycerol per cuvette (in 0.1-2.0 mL sample volume)

Read againt air or againt water (without a cuvette in the light path).

Table 12 Procedure for glycerol measurement

Pipette into cuvettes	Blank	Sample
distilled water (at 25°C)	2.00 mL	1.90 mL
sample	-	0.10 mL
buffer	0.20 mL	0.20 mL
solution 2 (NADH/ATP/PEP)	0.10 mL	0.10 mL
suspension 3 (PK/L-LDH)	0.02 mL	0.02 mL
Mix, read the absorbances of the solution (A_1) after aprox. 4 min (at complete of pre-reaction). Start the reactions by the addition of:		
suspension 4 (GK)	0.02 mL	0.02 mL
Mix, read the asorbances of the solution (A_2) at the end of the reaction (aprox. 5 min). If the reaction persists after 5 minutes, continue measuring the absorbances at 2-minute intervals until the absorbances remain constant for at least 2 consecutive minutes.		

Calculation:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{glycerol}$. The value of $\Delta A_{glycerol}$ should as a rule be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of glycerol can be calculated following the equation:

$$c = \frac{V \times MW}{\varepsilon \times d \times V} \times \Delta A_{glycerol} \quad [g/L]$$

where:

V = final valume [mL]

MW = molegular weight of glycerol [g/mol]

ε = exstinction coefficient of NADH at 340 nm

= 6300 [1 × mol^{-1} × cm^{-1}]

d = light path [cm]

v = sample valume [mL]

It follows for glycerol:

$$c = \frac{2.34 \times 92.1}{6300 \times 1.0 \times 0.1} \times \Delta A_{glycerol} \quad [g/L]$$

$$= 0.3421 \times \Delta A_{glycerol} \quad [g/L]$$

If the sample was diluted during preparation, the result must be multiplied by the dilution factor, denoted as F.

4.2.9 Total Phenolic Content

These compounds possess an aromatic ring and exhibit an absorption peak at 280 nm. The analysis involves determining the optical density (O.D.) at 280 nm in the beer.

Read the samples in 1 cm quartz cuvettes at 280 nm against water. Follow these steps at the spectrophotometer:

- Access Programs - Cary - Simple reads - Setup - Set wavelength to 280 - Confirm - Read (green light).
- Place cuvettes with water for zeroing.
- Zero the instrument.
- Insert the sample in the external cuvette.
- Express the result simply as O.D. 280 by multiplying the read value by the dilution used. This can be represented as mg/L of equivalent gallic acid (GAE) or as mg/L of hydrated catechin, after establishing a calibration curve using solutions with a known concentration in gallic acid or hydrated catechin.

In the latter case, by reading the absorbance at 280 nm and adhering to the Lambert-Beer law ($A = C * \varepsilon$), the trend of the function interpolating the values forms a straight line.
The angular coefficient of this line corresponds to ε and has a value of 0.146.

4.2.10 Antioxidant Power

The determination was conducted using the ABTS assay as described by (Jaramillo et al.2011). This spectrophotometric test assesses antioxidant capacity by measuring the ability to remove radicals from the ABTS+ cation (abbreviated for 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)). The radical is generated through the oxidation reaction of the colorless compound ABTS with potassium persulfate ($K_2S_2O_8$), as outlined by (Pellegrini et al.1999).
The resulting radical cation is highly stable and exhibits a vibrant blue-green color (with absorption maxima at 415, 645, 734, and 815 nm). Upon the addition of one or more antioxidants to the reaction environment, they donate one or more hydrogen atoms to the cationic radical, leading to a decrease in the initial absorbance value at λmax.
For the preparation of the reagent (utilizing glass bottles):
19.18 mg ABTS + 5 mL H_2O
3.33 mg $K_2S_2O_8$ + 88 µL H_2O

These two solutions are combined, and the resulting radical solution is stored in the dark at room temperature overnight. Subsequently, it is diluted with deionized water until the

absorbance value against the blank (deionized water) at λ734nm reaches 0.70±0.05. Next, 10 µL is added to 1 mL of the diluted radical solution. The absorbance value is monitored for 5 minutes, revealing a decrease from the initially reported value.
The final value is then compared with a Trolox standard, an analog of vitamin E, and the antioxidant activity is expressed in Trolox Equivalent Antioxidant Capacity (TEAC). The antioxidant activity of the samples is quantified through a Trolox dose-response curve within the concentration range of 0.2-1.5, expressed as TEAC/g.

10 mL of each beer were accurately measured and carefully placed separately into 30 mL glass flasks. The flasks were promptly covered with aluminum foil and allowed to stabilize at room temperature (approximately 24 °C) for one hour.

4.3 Volatilomic Analysis by SPME GC-MS

4.3.1 Sample Analysis: Isolation of VOCs

As recommended by the manufacturer's instructions, a preconditioned 100 µm polydimethylsiloxane beer was fitted to a manual sampling (Supelco, Bellefonte, PA, USA). The beer was then exposed to the headspace of the flask containing the samples for 30 min. By the end of the time, the sample was transferred into the GC-MS instrument for analysis. The process was carried out two times for each sample.

4.3.2 SPME GC-MS Analysis

Gas chromatography–electron impact mass spectrometry (GC–EIMS) analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS (Agilent Technologies Inc., Santa Clara, CA, USA) a capillary column (30 meters long with a diameter of 0.25 millimeters and a coating thickness of 0.25 micrometers) paired with an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). The experimental setup included the following conditions: the injector and transfer line temperatures were set to 220 °C and 240 °C, respectively; the oven temperature was programmed to increase from 60 °C to 240 °C at a rate of 3 °C per minute; helium gas was used as the carrier at a flow rate of 1 mL per minute; a 1 µL sample was injected with a split ratio of 1:25. For data acquisition, a full-scan mode was employed with a scan range of 30–300 m/z and a scan time of 1.0 second.

4.3.3 Identification of VOCs

Identification of the constituents was based on a comparison of the retention times with those of the beer samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons. Computer matching was also used against commercial (NIST 14 and ADAMS 07) and laboratory-developed mass spectra libraries built up of pure substances and components of known oils and MS literature data.

4.4 Sensory Analysis

Sensory analysis was performed at the sensory laboratory of the University of Pisa. Testing was carried out by a total of five individuals, ranging in age from 25 to 55 (2 women and 3 men), who were in good health and volunteered for the study. All assessors were affiliated with the University of Pisa, serving as regular staff, professors, and students, and possessed expertise in describing and critically evaluating aroma and taste. Participants did not receive any monetary compensation.

The tasting sessions occurred in a calm environment, free of strong odors at room temperature, lasting approximately 1 hour. The sample of five different beer samples (sugar, honey, red grape, white grape, and orange) were blindly submitted to the assessors and organized through a rotated tasting session. Beer glasses were labeled with 3 digits and served at a temperature of 10°C (10 ± 2°C). The tests were conducted between 12:00 and 13:00, with assessors instructed not to consume anything in the preceding 3 hours. All assessors received detailed instructions on the general procedure and guidelines for palate cleansing with water previously tested. Data collection was carried out using an online sensory sheet, filled through the the ISS platform.

4.5 Statistical analysis

The collected data underwent statistical analysis, and differences among means were assessed for significance using one-way ANOVA (CoStat, Version 6.451, CoHort Software, Pacific Grove, CA, USA).

Chemical analyses were conducted in triplicate and the data are reported as average values. Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$ significance was used for the separation of the samples.

Statistical analysis of volatile organic compounds included hierarchical cluster analysis (HCA) using the Ward method and employing two-way clustering, conducted using JMP Pro 17.0 software (SAS Institute, Cary, NC, USA).

The results of the sensory analysis were processed by the Big Sensory Soft 2.0 software (version 2018). Sensory data were analyzed by two-way ANOVA with panelists and samples taken as main factors, followed by the Friedman test to identify significant descriptors to discriminate samples.

5 Results and Discussion

5.1 Chemical Analysis

5.1.1 pH and Total Acidity

The pH and total acidity are considered the two most important criteria by the brewing industries which strongly influence sanitation and other physiological parameters like color odor, taste, and biological, and chemical stability. The brewing sector prioritizes pH and total acidity as crucial determinants that greatly impact sanitation and various physiological factors, including color, scent, taste, as well as biological and chemical stability. The optimal pH range of 3.90–4.20 for light lager beers, which holds significance throughout the brewing process. This range influences enzyme effectiveness, hop utilization, protein coagulation, and the monitoring of yeast activity during clean beer fermentation. Alcoholic beverages from Tanzania have been documented to exhibit pH and total acidity within the ranges of 3.9–5.5 and 0.41–0.062, 0.28–0.38, and 0.06–0.09 g/100 mL, respectively (Pai et al., 2015).

Beers with pH levels below 4 were labeled as 'high' (acidic), while those with pH levels exceeding 4.5 were designated as the lower acidic beers. The same trend in titratable acidity remained consistent across all the beers. Those with a pH below 4 exhibited titratable acidity levels surpassing 0.05 mol/L (Agorastos et al., 2023).

The results of the pH samples ranged from 4.64 to 4.72 (Figure 5A), meanwhile, the total acidity of the beer samples fell within the range of 0.024% to 0.029% tartaric acid equivalent (figure 5B). Although both results of pH and total acidity showed differences in number, there are no significant differences between samples.

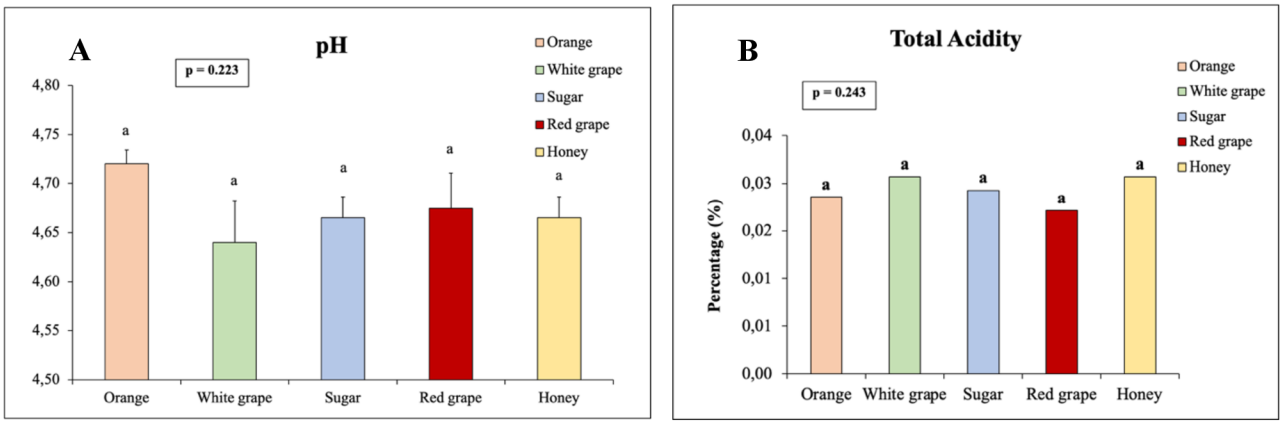


Figure 5 *pH values (A) and titratable acidity (B) evaluated in the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

5.1.2 Alcohol Content

The alcohol content of the beer sample ranged from 3.19% to 3.55%. The orange had the greatest content of alcohol followed by sugar, white grape, red grape, and honey (Figure 6).

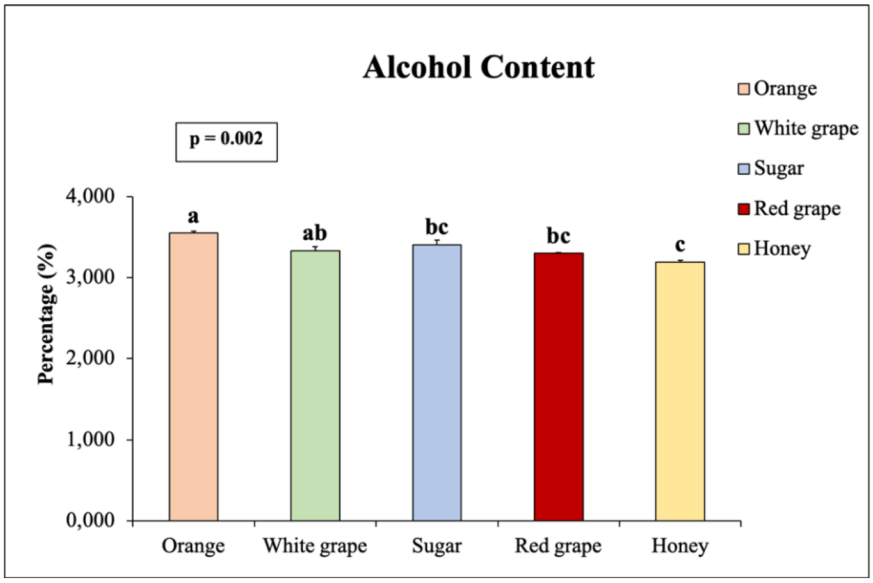


Figure 6 *Alcohol content (%v/v) of the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

Delving deeper into the statistical analysis, it became apparent that significant differences exist among the samples. Each substance exhibited distinct characteristics in terms of alcohol content, highlighting the variability inherent in these natural products. However, it is noteworthy that while sugar and red grapes showed notable differences from other samples, they did not display statistically significant distinctions from each other. These results added complexity to our understanding of the comparative alcohol content within these specific substances.

In this research, the alcohol content of beer samples fell within the range of 3.19% to 3.55%, categorized as low-alcohol beer. This range is similar to that of standard beers, as noted by (Missbach et al., 2017), which typically contain alcohol ranging from 3% to 6% by volume. Decreasing the alcohol content in beers leads to the production of beverages labeled as alcohol-reduced or alcohol-free beers (with ≤0.5% alcohol by volume). Earlier studies have indicated a growing global market share for alcohol-reduced and alcohol-free beers. Consequently, many prominent breweries have broadened their range to include beers with reduced alcohol content. Beer exhibits a remarkably intricate sensory profile (Brányik et al., 2012). The primary source of alcohol in beer stems from the process of fermentation. This transformative process unfolds

as yeast interacts with the sugars present in malted grains, predominantly barley, catalyzing their conversion into alcohol and carbon dioxide. Through this intricate chemical reaction, the sugars undergo fermentation, resulting in the production of ethyl alcohol-commonly known as ethanol, thus imbuing beer with its characteristic alcoholic content (Martinez et al., 2019).

5.1.3 Acetic Acid

Acetic acid plays a crucial role in beer by enhancing its flavor, aroma, and microbial resilience. This compound, known for its sour or vinegar-like taste, is generated during fermentation, primarily by specific bacteria such as acetobacter species. In this study, the concentration of acetic acid ranged from 0.003 g/L to 0.011 g/L. Notably, the honey sample displayed the highest level of acetic acid, followed by sugar, red grape, orange, and white grape. Statistical analysis revealed significant differences between most samples, except for orange, white grape, sugar, and honey, where no statistically significant distinctions were detected.

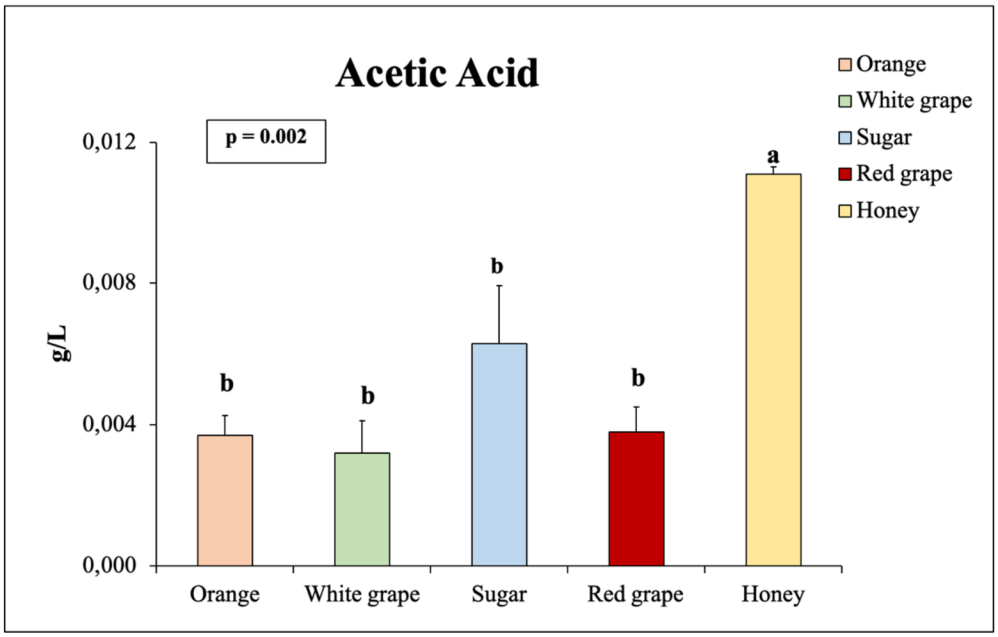


Figure 7 *Acetic acid content of beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

The results of this study highlight notable variations in acetic acid levels among the samples (Figure 7). Specifically, the honey sample displayed the highest acetic acid value, while the white grape sample showed the lowest acetic acid content compared to the other samples. Acetic acid, an organic acid present in beer, plays a role in shaping its flavor profile. It is primarily generated during fermentation as yeast metabolizes sugars. When present in small amounts, acetic acid contributes to a desirable tartness and adds complexity to the beer's flavor,

often enhancing other taste notes. However, elevated levels of acetic acid can result in off-flavors, such as a vinegary or sour taste, which may not be well-received by consumers. Brewers meticulously manage fermentation parameters to control the production of acetic acid, ensuring a balanced and enjoyable beer experience for consumers (Dysvik, Leanti, et al., 2020). The acetic acid content in beer is subject to influence from several key factors during the brewing process. These include fermentation conditions, oxygen exposure, and conditions during beer aging (Dysvik, La Rosa, et al., 2020).

5.1.4 D-Glucose and D-Fructose

D-glucose and D-fructose are vital components in beer manufacturing, playing key roles in shaping its taste, texture, and overall excellence. D-glucose, also referred to as glucose or sugar, is a fermentable sugar found in wort, which is the liquid obtained from malted grains during brewing. Similarly, D-fructose, another fermentable sugar, is present in wort and aids in the fermentation process. The analysis of D-glucose content in the beer samples revealed a range spanning from 0.002 g/L to 0.013 g/L (Figure 8A). Interestingly, the sugar sample demonstrated the highest D-glucose content, followed by the orange, white grape, honey, and red grape samples. Statistical analysis indicated significant differences between most samples, except for the orange, white grape, and sugar samples, where no statistically significant distinctions were found. The D-fructose content in the beer samples exhibited a range from 0.0 g/L to 0.024 g/L (Figure 8B). Remarkably, the sugar sample demonstrated the highest D-fructose content, followed by red grape, white grape, and orange samples. Notably, D-fructose was not detected in the honey sample.

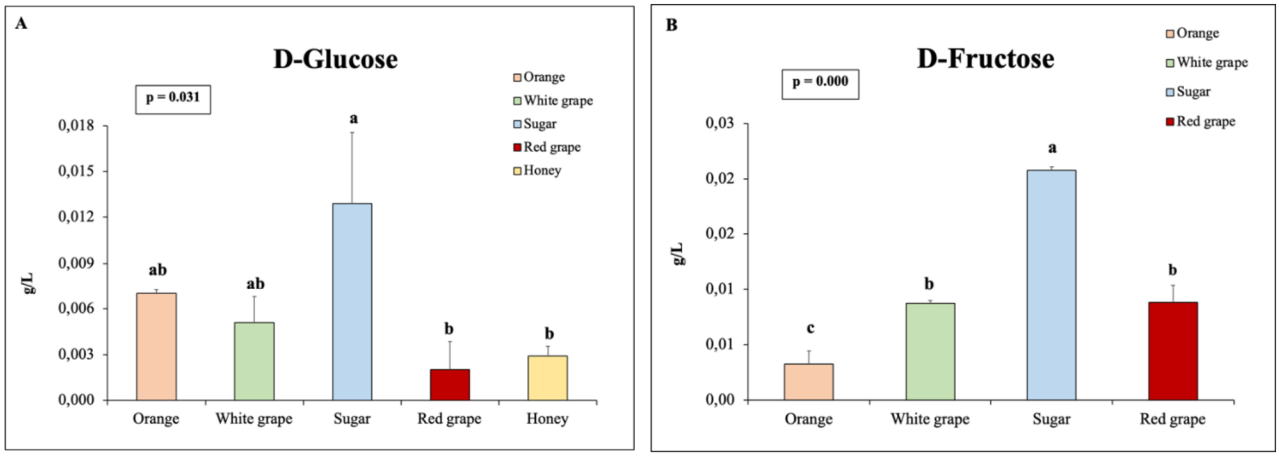


Figure 8 *D-glucose (A) and D-fructose (B) content of beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

Adjuncts might be incorporated to increase fermentable sugars in specific beer varieties, consequently impacting the levels of D-glucose and D-fructose. It is imperative to oversee and regulate these sugars to attain the intended flavor profiles and characteristics in the finished beer. D-fructose is an isomer of D-glucose, and is widely used in the food industry. It is naturally found in various fruits such as grapes, bananas, strawberries, oranges, and apples. This monosaccharide is a constituent of sucrose and possesses high sweetness, being approximately 140% as sweet as sucrose (Shintani, 2019). In beer brewing, D-glucose and D-fructose are vital components that contribute to the fermentation process and the overall flavor profile of the finished product (Ciosek, Fulara, et al., 2020)

5.1.5 Total Lactic Acid

Lactic acid serves as a pivotal element in the souring process, notably in beer styles like sour ales and lambics. Its inclusion introduces a tangy, sour flavor profile to the beer, varying in intensity based on brewing techniques and fermentation variables. Moreover, lactic acid is instrumental in regulating the beer's pH level. As fermentation progresses, the production of lactic acid aids in pH reduction, impacting factors like yeast performance, microbial preservation, and the beer's overall sensory attributes. In this study, the concentration of total lactic acid ranged from 0.011 g/L to 0.032 g/L. Notably, the white grape sample displayed the highest level of total lactic acid, followed by orange, red grape, sugar, and honey. Statistical analysis revealed significant differences between most samples, except for orange, white grape, where no statistically significant distinctions were detected (Figure 9).

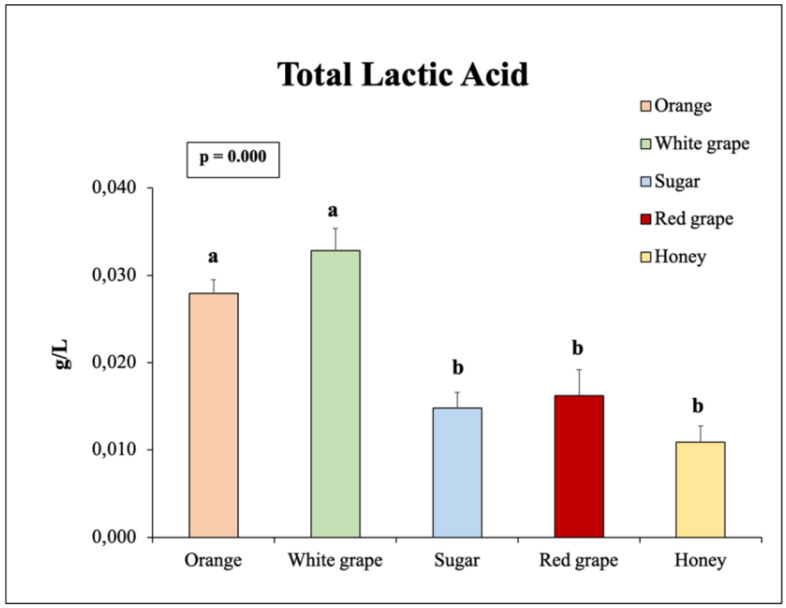


Figure 9 *Total lactic acid content of beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

Specifically, the white grape sample displayed the highest acetic acid value, while the honey sample showed the lowest total lactic acid content compared to the other samples. The main product of lactic acid bacteria is lactic acid, a significant component in sour beers. Brewers commonly gauge the lactic acid concentration in beer by assessing its pH, presuming that lower pH levels correspond to higher lactic acid content (Ciosek, Rusiecka, et al., 2020). Lactic acid is vital in crafting sour beers, adding to their characteristic tangy and acidic taste profiles. In beer styles like Berliner Weisse, Gose, and select Lambics, brewers intentionally incorporate lactic acid to introduce a refreshing sourness and enhance the beer's complexity (Fu et al., 2024).

5.1.6 Glycerol

Glycerol is a crucial element in beer manufacturing, influencing its flavor, mouthfeel, and stability. One major effect of glycerol in beer is its influence on mouthfeel, where it contributes to the beer's body and thickness, creating a smooth and substantial sensation. This enriches the overall enjoyment of the beer by delivering a satisfying and luxurious texture. The analysis of glycerol content in the beer samples revealed a range spanning from 1.37 g/L to 1.70 g/L. Statistical analysis indicated no statistically significant differences were found (Figure 10).

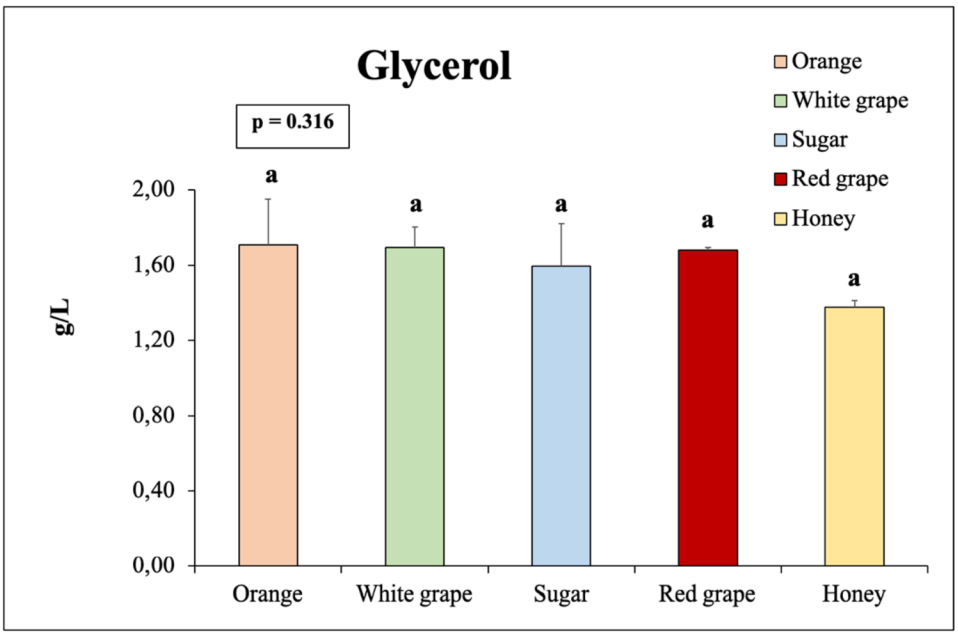


Figure 10 *Glycerol content of beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

In this study, the results indicate that the glycerol values across all samples is largely consistent. However, it was noted that the orange sample exhibited higher content of glycerol, while honey has the lowest content of glycerol among samples. Glycerol, also referred to as glycerin, is a natural component present in beer, often generated by yeast as a metabolic byproduct during fermentation. It enhances the mouthfeel and body of the beer, lending it a smooth and mildly viscous texture. When present in moderate quantities, glycerol can improve the overall sensory perception by contributing to the beer's sense of fullness and smoothness. However, an excessive presence of glycerol may lead to a beer with an overly thick or heavy sensation on the palate (Hlangwani et al., 2024).

5.1.7 Total Phenolic Content

Beer constitutes a notable source of phenolic compounds, which actively influence its taste, appearance, and overall sensory experience. Moreover, these compounds are believed to play a crucial role in assuring the antioxidant potential of beer and may contribute significantly to maintaining the internal redox balance within the human body. Phenolic compounds are believed to originate from polyphenols found in plants. The total phenolic content of the beer samples fell within a range of 2.084 mg/mL to 2.127 mg/L (Figure 11).

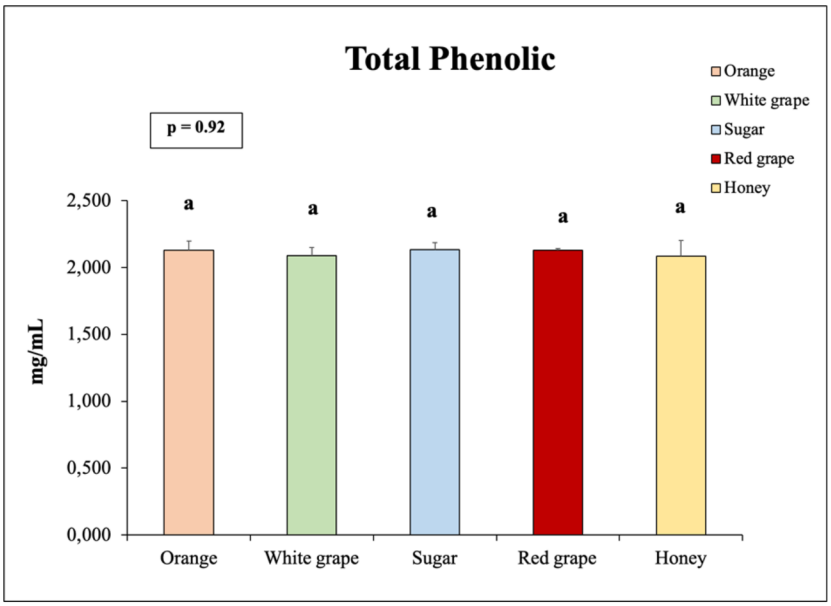


Figure 11 *Total phenol content (mg/mL of gallic acid) of the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

While discernible differences were observed among the samples, statistical analysis revealed that these variations did not reach significance.

The study conducted by (Nardini, 2023) shows that TPC are present in various fruits, vegetables, and honey; Nardini reports that grape samples ranged from 2.19 mg/L to 4.67 mg/L, which aligns closely with the results obtained from our grape samples. Furthermore, orange peel samples exhibited a range of 3.83 mg/L to 4.82 mg/L, whereas honey samples ranged from 3.82 mg/L to 4.46 mg/L. These values are notably higher compared to our study, likely due to variations in orange varieties and honey samples. Polyphenols act as natural antioxidants and influence the quality of beers, additional analyses are required to establish the composition of phenolic acids, flavonoids, and the stilbene derivative resveratrol. The study also measured hydroxycinnamic acid derivatives including chlorogenic, neochlorogenic, vanillic, caffeic, p-coumaric, and ferulic acids, as well as hydroxybenzoic acid derivatives such as syringic and sinapic acids. Furthermore, the study evaluated flavonoids such as catechin, rutin, myricetin, and quercetin, in addition to the stilbene derivative resveratrol. These compounds have the potential to improve both the chemical and sensory characteristics of beer (Nardini & Garaguso, 2020).

5.1.8 Antioxidant Power

The primary antioxidants found in beer encompass phenolic compounds, melanoidins, sulfur dioxide (SO_2), vitamins, among others. The quantities of these components are significantly influenced by both genetic and agricultural variables impacting the raw materials, as well as the technological aspects inherent in the brewing procedures. The antioxidant power levels in this study varied between 1.16 TEAC/g and 1.48 TEAC/g across the samples. Remarkably, the honey sample exhibited the highest antioxidant power content, closely trailed by white grape, sugar, orange, and red grape, in descending order. Notably, statistical analysis revealed significant differences between most samples, with the exception of the honey and white grape samples. Honey sample has the highest content of antioxidant capacity, while red grape has the lowest content of antioxidant capacity among all samples.

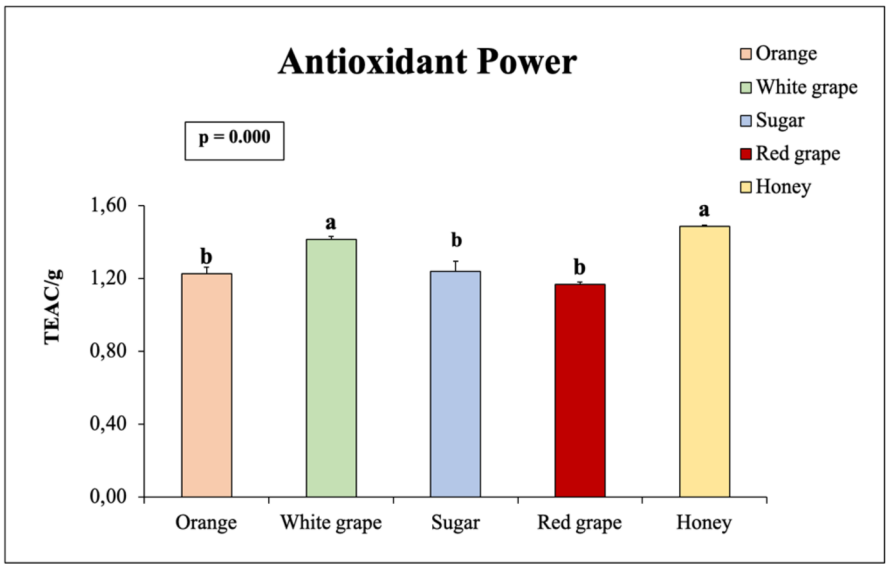


Figure 12 *Antioxidant power (TEAC/g) of the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

The study conducted (Nardini, 2023) highlights the antioxidant activity present in various fruits, vegetables, and honey. The summarized antioxidant capacity in this study reveals that grape samples ranged from 1.5 TEAC/g to 2.0 TEAC/g, similarly, orange peel samples ranged from 1.5 TEAC/g to 2.0 TEAC/g, while honey samples ranged from 1.5 TEAC/g1 to 2.6 TEAC/g. The results of this study indicate slightly higher values in comparison to our research, which could be attributed to differences in fruit varieties. It is noteworthy that the majority of beers containing fruit adjuncts demonstrate elevated levels of polyphenols, enhanced antioxidant properties, improved fragrance, and increased content of volatile compounds (Gasiński et al., 2022c).

5.1.9 Beer Bitterness

The analytical assessment of bitterness in all beer samples was conducted, with the composition of hops used serving as the basis for this determination. This careful analysis underscores the crucial role that different hop varieties and their unique aromas play in shaping the perceived intensity of bitterness in beer. Indeed, the intricate interplay between hop selection and aroma composition can greatly impact the overall sensory experience of consuming a particular brew. The bitterness value of the beer samples ranged from 11.16 EBU to 14.32 EBU (Figure 13). Remarkably, the red grape beer showed the maximal value followed by honey, sugar, orange, and white grape. Based on the results, there is a statistically significant

differences between the samples, while red grape and honey are found to be no statically significant differences between samples.

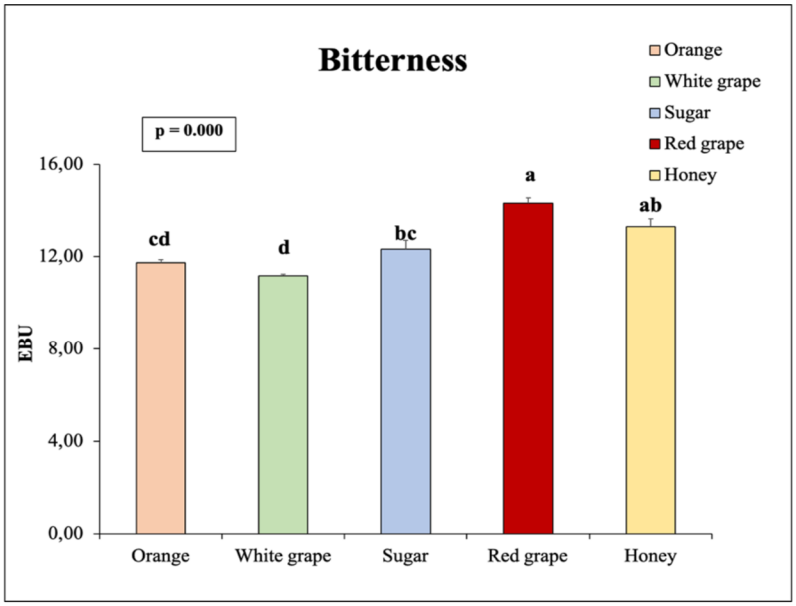


Figure 13 *Bitterness values (EBU) evaluated in the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

The bitterness perceived in beer originates from a broader spectrum of compounds beyond solely iso-α-acids. While compounds such as β-acids, humulinones, hulupones, hard resins, and polyphenols exhibit lower bitterness levels and are found in hops in lesser amounts compared to α-acids, they can still contribute, in conjunction with α-acids, to the ultimate bitterness profile of beer. Several factors, including fermentation, pH levels, boiling, dry hopping, and beer aging (storage), significantly contribute to reducing the perceived bitterness. (Klimczak & Cioch-Skoneczny el al., 2023). The authors (Aaron Justus el al., 2018) observed an average decrease of 33.7% in IBU among 14 brewed beers. They highlighted that beers relying heavily on whirlpool hopping experience even greater reductions in bitterness. This phenomenon might stem from the increased formation of trans isomers during this specific hopping process.

5.1.10 Color of beer

The use of malted or unmalted wheat and others adjunts can influence beer colour. Visual cues, such as color, provide cues that shape expectations regarding the taste and flavor characteristics of food and beverages especially beer. The color units ranged from 19.94 EBC to 31.11 EBC across the samples (Figure 14). Red grape exhibited the highest color unit value,

succeeded by honey, sugar, orange, and white grape. The results indicate statistically significant differences among the samples.

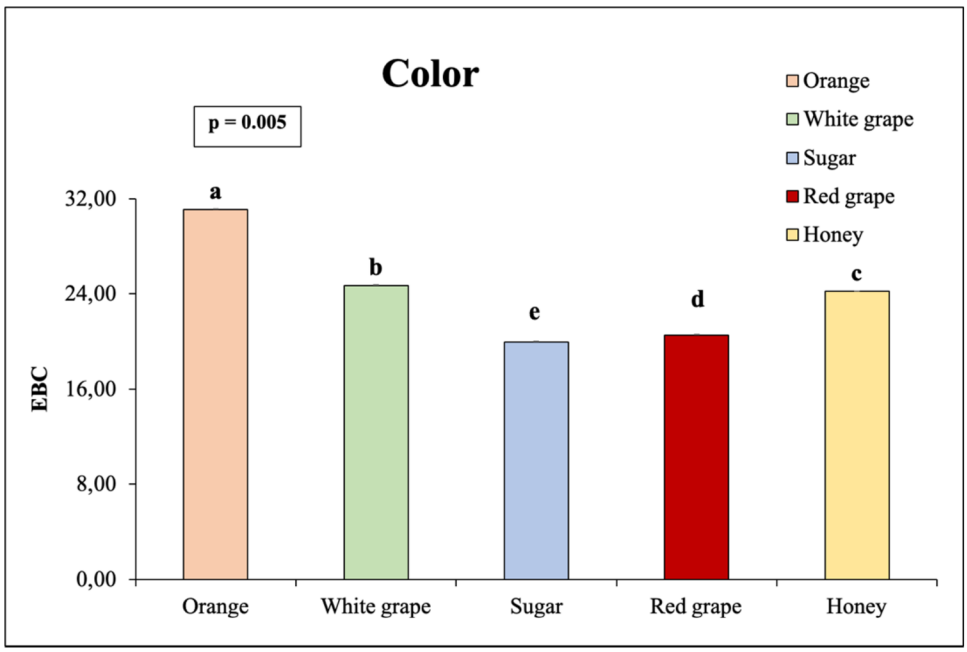


Figure 14 *EBC color evaluated in the beer samples; values shown represent the arithmetic mean (n=3); error bar indicates standard diviation; lowercase letters indicate statistically significant differences between samples*

Table 13 reports the CieLab coordinates detected in beer samples, using a tristimulus colorimeter.

Table 13 CieLab coordinates detected in beer samples

	L*	a*	b*	C*	h*
Orange	52.58^{a}	-0.41^{ab}	12.34^{a}	12.35^{a}	-88.10^{a}
White Grape	51.79^{a}	-0.37^{ab}	12.71^{a}	12.72^{a}	-88.33^{a}
Sugar	52.17^{a}	-0.86^{b}	13.48^{a}	13.51^{a}	-86.35^{a}
Red Grape	51.79^{a}	0.50^{a}	13.33^{a}	13.33^{a}	-87.84^{a}
Honey	52.58^{a}	-0.86^{ab}	13.39^{a}	13.40^{a}	-87.78^{a}

In order to evaluate the color differences between sample, the ΔE was considered. The highest the delta E, the highest the difference between the color of samples.

The values used to determine whether the total colour difference was visually obvious were the following:

ΔE < 1—color differences are not noticeable by the human eye.

1 < ΔE < 3—color differences are not obvious to the human eye.

ΔE > 3—color differences are obvious to the human eye.

Table 14 Color difference between samples according to ΔE

ΔE	Orange	White Grape	Sugar	Red Grape	Honey
Orange		0.68	1.84	1.92	1.09
White Grape			0.56	2.62	0.75
Sugar				1.08	0.97
Red Grape					1.68
Honey					

Based on the ΔE results shown in Table 14, the orange sample exhibits the highest ΔE value. There appears to be no discernible color difference between the orange and white grape samples. For the remaining samples, any color differences are not readily apparent to the human eye.

The results of this study indicate considerable diversity in beer color levels across the samples. Particularly, the orange sample exhibits the highest color value, whereas the sugar sample displays the lowest value among all beer samples. The color in beer extends beyond mere aesthetics; it serves as a key indicator of various attributes such as malt type, brewing process, and even flavor profile. The color of beer can influence consumer perception, with darker hues often associated with richer, more complex flavors, while lighter colors may suggest a crisper or more refreshing taste. Additionally, color can provide insight into the beer's ingredients and potential brewing faults. In essence, the color of beer plays a significant role in both sensory experience and overall quality assessment (Prado et al., 2021). Beer color is affected by malt type and quantity, roasting level, brewing processes, adjuncts like sugars or fruits, hopping methods, water composition, yeast strain, and oxidation levels. Darker malts and extended roasting yield deeper colors, while lighter malts produce lighter hues. Brewers can manipulate color with various processes and ingredients, resulting in a wide range of beer styles (Van Doorn et al., 2019).

5.2 Volatilomic Analysis by SPME GC-MS

In this study, a comprehensive analysis identified a diverse array of 53 volatile compounds present in beers, with esters, alcohols, and acids emerging as the primary constituents dictating the aromatic character. These compounds collectively contribute to the intricate tapestry of aromas that define each brew. Moreover, the study highlighted the significant role of various adjuncts in shaping the nuanced aroma profiles observed in the beers examined. Among these volatile compounds, esters stood out as particularly noteworthy, being predominantly volatile in nature and synthesized during the fermentation process. Notably, the beer samples revealed

the presence of 15 distinct ester compounds, with notable mentions including ethyl acetate, ethyl hexanoate, and isoamyl acetate, each imparting its unique aromatic signature to the brew. Additionally, volatile alcohols emerged as notable byproducts of amino acid metabolism during fermentation, with ethanol, 3-methyl-1-butanol, and 2-methyl-1-butanol being prominent examples. Despite their relatively low quantitative presence, volatile acids proved to be indispensable contributors to the overall sensory experience of the beers, playing crucial roles in acidity modulation, aroma enhancement, and foam stability. Noteworthy among these were hexanoic, octanoic, and nonanoic acids, detected in small quantities within the beer samples, yet exerting profound effects on the sensory attributes of the final product.

Table 15 Volatilome in beers: orange (A), white grape (B), sugar (M), red grape (R), and honey (Z)

Compounds	Class	LRI	A	DevSt	B	DevSt	M	DevSt	R	DevSt	Z	DevSt
ethanol	nt	427	6,3	0,68	13,4	1,19	12,0	0,50	14,4	0,83	11,5	0,87
n-hexane	nt	600	0,0	0,00	0,0	0,00	0,8	0,24	0,0	0,00	0,0	0,00
ethyl acetate	nt	611	1,3	0,40	4,8	1,24	3,2	0,01	5,7	2,17	2,8	0,16
3-methyl-1-butanol	nt	736	1,6	0,59	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
isopenthyl alcohol (=1-butanol, 3-methyl)	nt	736	1,3	0,32	8,2	0,40	6,3	0,50	8,3	0,28	6,8	0,19
2-methyl-1-butanol	nt	739	3,4	0,37	3,5	0,98	4,0	0,17	3,9	0,05	3,5	0,41
isobutyl acetate	nt	771	0,0	0,00	0,4	0,14	0,3	0,02	0,3	0,03	0,2	0,02
ethyl butyrate	nt	802	0,0	0,00	0,2	0,03	0,2	0,00	0,2	0,02	0,2	0,02
isoamyl acetate (=isopenthyl acetate= 1-butanol, 3-methyl, acetate	nt	876	6,7	0,30	13,3	0,80	11,6	0,68	14,5	0,53	10,9	0,01
α-thujene	mh	933	0,0	0,00	0,0	0,00	0,1	0,11	0,0	0,00	0,0	0,00
α-pinene	mh	941	0,0	0,00	0,0	0,00	0,8	0,22	0,0	0,00	0,0	0,00
camphene	mh	952	0,0	0,00	0,0	0,00	0,3	0,06	0,0	0,00	0,0	0,00
pentyl prppanoate (=amyl propionate	nt	969	0,0	0,00	0,1	0,11	0,0	0,00	0,0	0,00	0,0	0,00
pentyl prppanoate (=amyl propionate	nt	969	0,0	0,00	0,1	0,09	0,2	0,01	0,2	0,00	0,0	0,00
β-pinene	mh	982	0,0	0,00	0,0	0,00	0,6	0,06	0,2	0,02	0,0	0,00
hexanoic acid	nt	988	0,0	0,00	0,2	0,15	0,0	0,00	0,0	0,00	0,1	0,12
myrcene	mh	991	1,3	0,03	0,3	0,01	1,5	0,58	0,4	0,02	1,5	0,50
ethyl hexanoate	nt	1000	6,4	0,71	6,8	0,31	5,5	0,32	6,3	0,19	5,5	0,29
3-carene	mh	1011	0,0	0,00	0,0	0,00	0,4	0,06	0,0	0,00	0,0	0,00
α-terpinene	mh	1020	0,0	0,00	0,0	0,00	0,1	0,08	0,0	0,00	0,0	0,00
p-cymene	mh	1028	0,2	0,05	0,0	0,00	2,5	0,60	0,0	0,00	0,0	0,00
eucalyptol	om	1031	0,0	0,00	0,0	0,00	0,3	0,08	0,0	0,00	0,0	0,00
limonene	mh	1032	27,2	1,08	0,0	0,00	5,5	0,81	0,0	0,00	0,0	0,00
γ-terpinene	mh	1060	1,1	0,03	0,0	0,00	0,6	0,15	0,0	0,00	0,0	0,00
1-octanol	nt	1071	1,8	0,01	0,2	0,02	0,2	0,03	0,2	0,02	0,2	0,00
ethyl heptanoate (=heptanoic acid, ethyl ester	nt	1097	0,0	0,00	0,2	0,00	0,2	0,02	0,2	0,01	0,2	0,01

linalool	om	1101	1,7	0,01	1,3	0,16	1,2	0,09	1,2	0,07	1,0	0,02
nonanal	nt	1102	0,0	0,00	0,0	0,00	0,1	0,15	0,2	0,01	0,4	0,12
phenylethyl alcohol (=phenetol)	nt	1116	7,3	0,21	15,2	0,28	13,8	0,91	15,0	0,38	13,5	0,11
camphor	om	1145	0,0	0,00	0,0	0,00	0,2	0,00	0,0	0,00	0,0	0,00
4-terpineol	om	1177	0,3	0,04	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
octanoic acid	nt	1180	0,2	0,04	1,0	0,19	0,9	0,18	1,0	0,10	0,8	0,18
α-terpineol	om	1189	0,4	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
ethyl octanoate	nt	1197	15,2	0,54	12,3	1,10	12,3	0,32	11,2	1,01	17,1	1,15
decanal	nt	1206	0,0	0,00	0,0	0,00	0,1	0,08	0,0	0,00	0,0	0,00
1-octyl acetate	nt	1213	0,4	0,01	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
citronellol	om	1228	1,0	0,04	0,4	0,03	0,3	0,03	0,3	0,02	0,3	0,01
2-phenylethyl acetate (=acetic acid, 2-phenylethyl ester	nt	1258	1,5	0,06	3,2	0,20	3,2	0,26	3,7	0,12	3,5	0,01
1-decanol	nt	1273	0,5	0,06	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
ethyl nonanoate	nt	1296	0,0	0,00	0,1	0,13	0,0	0,00	0,0	0,00	0,0	0,00
nonanoic acid, ethyl ester	nt	1296	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	1,0	0,15
nonanoic acid, ethyl ester (=wine ether=ethyl nonanoate	nt	1296	0,0	0,00	0,0	0,00	0,1	0,09	0,1	0,09	0,0	0,00
trans-geranic acid methyl ester (=methylgeranoate	nt	1324	0,0	0,00	0,0	0,00	0,3	0,02	0,3	0,01	0,5	0,03
methyl geranate		1325	0,1	0,05	0,3	0,02	0,0	0,00	0,0	0,00	0,0	0,00
cytronellyl acetate	nt	1350	0,5	0,01	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00
ethyl 9-ddecenoate	nt	1387	1,9	0,03	2,2	0,27	2,2	0,00	2,0	0,27	3,0	0,02
ethyl decanoate	nt	1396	7,0	0,14	7,7	0,51	5,7	0,26	5,6	0,98	10,9	0,35
β-caryophyllene (=E-caryophyllene)	sh	1419	0,0	0,00	0,0	0,00	0,1	0,07	0,0	0,00	0,1	0,11
octanoic acid, 3-methylbutyl ester (=isopentyl octanoate	nt	1446	0,0	0,00	0,0	0,00	0,0	0,00	0,0	0,00	0,1	0,10
α-humulene	sh	1454	0,0	0,00	0,0	0,00	0,1	0,12	0,0	0,00	0,6	0,07
ethyl dodecanoate	nt	1595	1,5	0,01	1,7	0,06	0,6	0,04	1,1	0,21	1,6	0,04
oxime, methoxy-phenyl			0,8	0,12	0,8	0,27	0,8	0,02	0,8	0,04	0,6	0,03
silanediol, dimethyl	nt-s		0,5	0,04	1,6	0,21	0,9	0,04	2,4	0,92	1,0	0,04
Total Identified			99,4		99,2		99,8		99,4		99,4	

To obtain deeper understanding regarding the factors influencing the variation in volatile compound concentrations within the beer samples, Hierarchical Cluster Analysis (HCA) was conducted based on the correlation matrix. The results depicted in Figure 15 illustrate the correlation of volatile compounds obtained from SPME GC-MS. Notably, the data indicates a distinct separation between sample A and the others, suggesting significant dissimilarity. Samples B and R appear closely clustered, indicating a very similar volatile composition, while there is also a discernible resemblance between samples M and Z. The methodology employed yields practical insights, not particularly evident in the differentiation between samples B and R, which represent distinct grape varieties. Meanwhile, samples M and Z, representing sugar and honey respectively, exhibit a structural similarity owing to their

shared composition primarily comprising sugar molecules. This underscores the method's effectiveness in discerning nuances even amidst varying sample types, shedding light on their underlying chemical compositions.

In a recent study conducted by (Mastrangelo et al., 2023) focusing on Italian grape ale (IGA) beer, intriguing parallels were observed with our investigation focusing on the volatile compounds present in grape samples. This alignment in this study underscores the consistency and relevance of our research within the broader context of understanding grape-derived beers. Adjuncts, such as fruits, encompass a rich array of volatile compounds closely tied to aroma perception. Among these compounds are esters, alcohols, acids, terpenoids, sulfides, and carbonyl compounds, each playing a significant role in shaping the aromatic profile of the final product. Their diverse presence highlights the complexity and potential for nuanced sensory experiences within beverages enhanced by such adjuncts (Rodriguez-Bencomo et al., 2012).

The gas chromatographic analysis shows that the formulation adopted, led to the production of different VOC profiles, as highlighted also by the HCA analysis (Figure 15). In particular, the ingredients used strongly influenced the volatile expression, allowing clear grouping of the samples into four separate clusters.

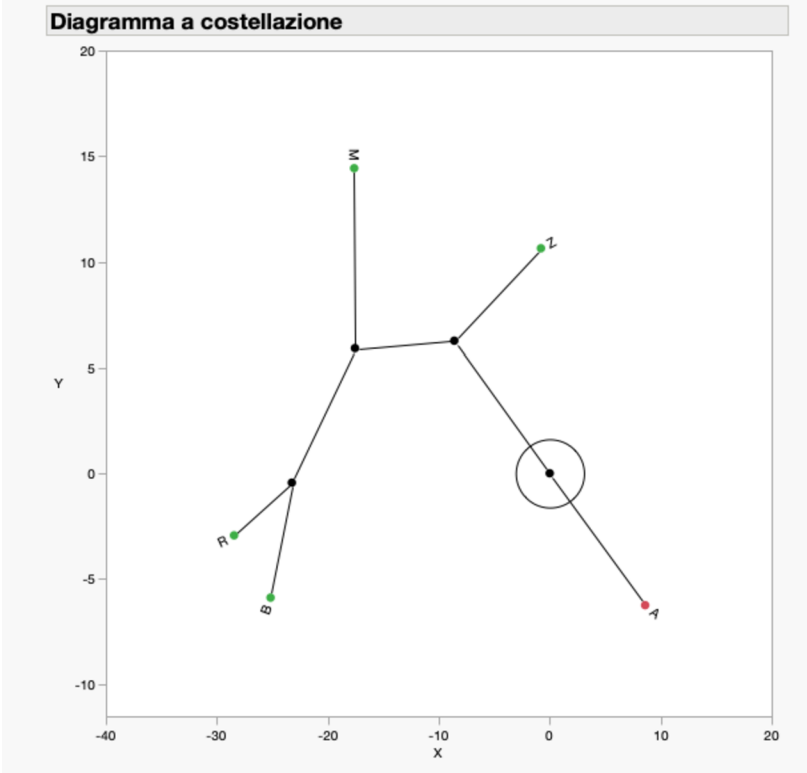


Figure 15 *Hierarchical Cluster Analysis (HCA) of groups of volatile compounds and beers sensory descriptors: orange peels (A), white grape (B), sugar (M), red grape (R), and honey(Z)*

5.3 Sensory Analysis

The sensory evaluation in this study involved five assessors and was conducted at the sensory laboratory of the University of Pisa. The evaluation process utilized an analytical program (Big Sensory Soft 2.0) for statistical analysis. Figure 10(A), (B), and (C) present spider plots showing the visual, aroma, and taste characteristics of the beer samples. Statistically significant differences were noted among the samples regarding visual attributes, encompassing color intensity, pale, gold, amber, brilliance, thin foam, compact foam, and foam persistency. Notably, the sensory evaluation aligned with the color results derived from chemical analysis, confirming consistency across both methodologies. Regarding aroma attributes, no statistically significant differences were observed between samples in terms of tropical, flowery, and spice aromas. However, significant differences emerged in the frankness of smell, citrus, red fruit, vegetal notes, smell oxidation, and intensity of aroma among the samples. Similarly, no statistically significant differences were detected between samples in terms of the frankness of taste, fineness, mineral content, and taste persistence attributes. Moreover, significant differences were found in the intensity of taste, bitterness, astringency, smoothness, and carbonation attributes.

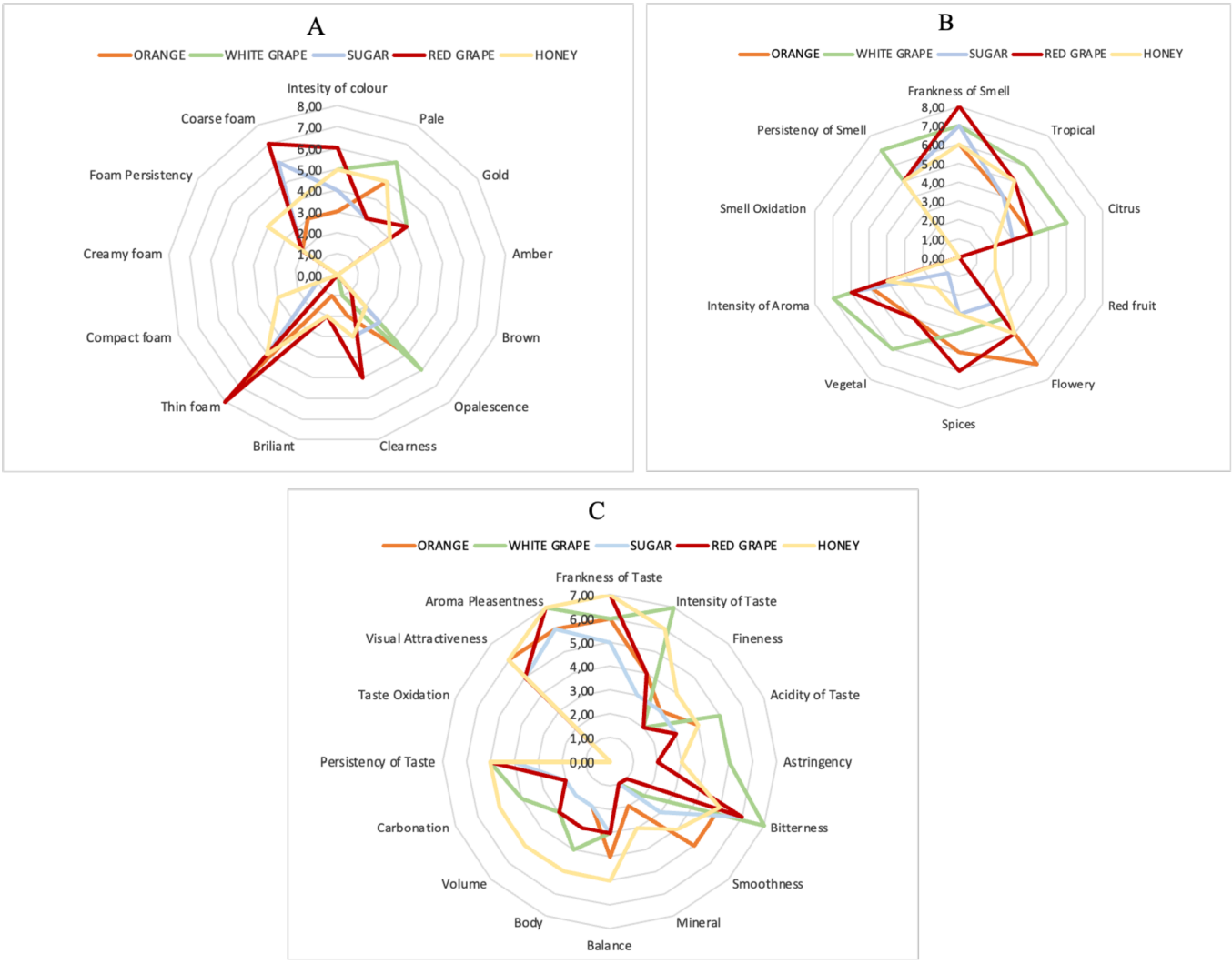


Figure 16 *Spider plots of visual (A), aroma (B), and taste (C) descriptors indicated by panelists; values shown represent the arithmetic mean (n=3)*

The comprehensive analysis incorporating feedback from all assessors (Figure 17) revealed a clear preference for honey beer, which stood out as the favored option. Following closely behind were white grape, orange, red grape, and sugar, in a descending order reflecting their respective levels of preference. This hierarchical ordering not only underscores the nuanced distinctions in taste preferences but also provides valuable insights into the diverse sensory perceptions of the beer samples which is crucial for this study.

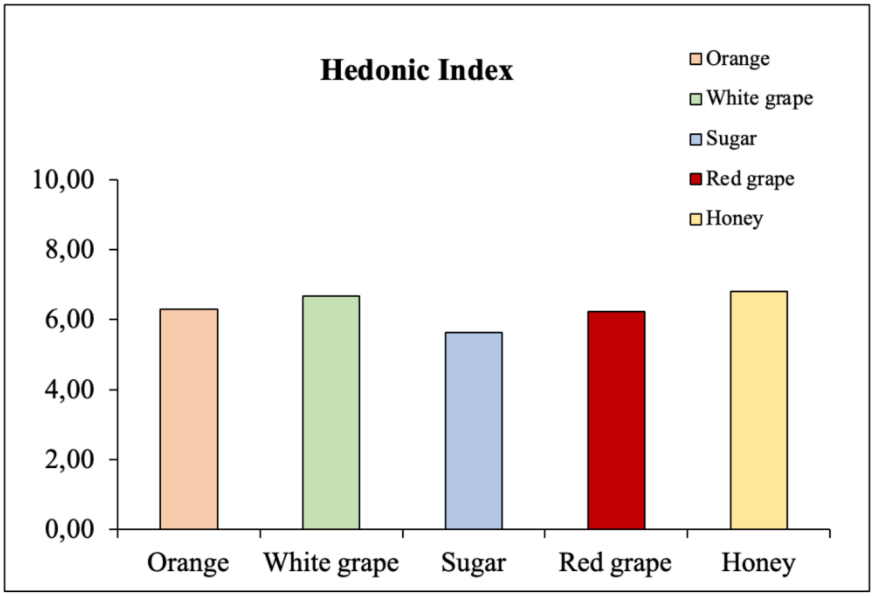


Figure 17 *The hedonic index of the beer samples; values shown represent the arithmetic mean (n=3)*

The sensory analysis unveiled that the current samples primarily impacted the intended attributes, with no unforeseen or undesirable flavor traits identified. Specifically, in Figure 17, it is evident that the white grape sample exhibits a more robust aroma profile characterized by tropical, citrus, and vegetal notes, along with enhanced aroma persistence. This observation aligns with the volatile compound results presented in Table 15, where the orange sample displays elevated levels of ethyl hexanoate, isoamyl acetate, and isopentyl alcohol. Table 12 shows the relationship between volatile compounds and the sensory attributes of the beer samples. Ethanol exhibits a positive correlation with the intensity of aroma, pleasantness, and frankness of smell, along with imparting tropical notes.

Table 16 Correlations between volatile compounds and sensory profile of beer samples

	ethanol	n-hexane	ethyl acetate	3-methyl-1-butanol	isopenthyl alcohol (=1-butanol, 3-methyl)	2-methyl-1-butanol	isobutyl acetate	ethyl butyrate	isoamyl acetate (=isopenthyl acetate= 1-butanol, 3-methyl, acetate	α-thujene
Frankness of Smell	0,774	0,1336	0,8927	-0,5345	0,6659	0,7299	0,6699	0,5345	0,8313	0,1336
Tropical	0,631	-0,5345	0,6678	-0,5345	0,7076	-0,2654	0,6699	0,5345	0,641	-0,5345
Citrus	0,1785	-0,3015	0,4255	0,0754	0,1578	-0,2121	0,3779	-0,0754	0,2599	-0,3015
Red fruit	-0,0036	-0,25	-0,246	-0,25	0,1211	-0,331	-0,1474	0,25	-0,0937	-0,25
Flowery	-0,714	-0,6784	-0,4918	0,8292	-0,7076	-0,6488	-0,8447	-0,8292	-0,661	-0,6784
Spices	0,0049	-0,5145	0,3486	0,343	-0,0924	0,0284	-0,177	-0,343	0,1157	-0,5145
Vegetal	0,0965	-0,6882	0,3772	0,1721	0,1093	-0,4842	0,186	-0,1721	0,1806	-0,6882
Intensity of Aroma	0,4994	0,1961	0,6373	-0,2942	0,4335	0,3408	0,694	0,2942	0,5586	0,1961
Persistency of Smell	0,3353	-0,25	0,4013	-0,25	0,3944	-0,331	0,5898	0,25	0,356	-0,25
Aroma Pleasentness	0,6902	-0,6124	0,6924	-0,6124	0,7589	-0,1351	0,5417	0,6124	0,6885	-0,6124
Overall Pleasantness	-0,0713	-0,9186	-0,0053	0,1021	0,0303	-0,7095	-0,2408	-0,1021	-0,0688	-0,9186

Conversely, ethyl acetate demonstrates positive correlations with tropical, citrus, spice, and vegetal characteristics. Additionally, 3-methyl-1-butanol is notably associated with flowery aroma perception. The tactile sensation experienced in the mouth when consuming any beer serves as a crucial gauge of consumer acceptance and preference, and this holds for beer as well (Fox et al., 2022). The study conducted by (Qi et al., 2024) indicates the utilization of grape adjuncts in beer brewing, elucidating the multifaceted ways in which these supplementary ingredients influence the beer's flavor, aroma, mouthfeel, and appearance. This exploration is rooted in the recognition of the diverse array of chemical compounds present in adjuncts, underscoring their potential to significantly impact the sensory profile of the final brew.

6 Conclusions

The use of fruit adjuncts like grape, and grape must into the brewing processes to provide fermentable carbohydrates for yeast. This incorporation of adjuncts, either alongside or instead of barley malt, serves several purposes, including improving local market accessibility, altering the sensory profile of the beer, and notably, reducing production costs. This study explores the various impacts of operating conditions and formulation on beer, with a specific focus on the incorporation of five different adjuncts: orange peel, red grape, white grape, sugar, and honey.

The chemical analysis of all beer samples reveals intriguing findings, notably showing elevated levels of polyphenols, predominantly phenolic acids, aromatic compounds, and significant antioxidant capacity. Interestingly, the total phenolic content remains largely consistent across all samples. However, the antioxidant power exhibits slight variability among the samples. Specifically, the honey sample demonstrates the highest antioxidant capacity, whereas the red grape sample exhibits the lowest among all samples. Volatile compounds obtained from SPME GC-MS indicate the varieties of volatile compounds present in beer samples, which is closely related to the beer sensory profile. The sensory analysis, incorporating input from all assessors, unveiled a distinct preference for honey beer, which emerged as the top choice. Following closely were white grape, orange, red grape, and sugar, ranked in descending order based on their respective levels of preference.

The potential outcomes of this study present numerous fascinating paths for ongoing research and investigation. These include exploring advanced analytical methods, optimizing operational parameters, conducting microbiological studies, and delving into consumer perception, all aimed at identifying new alternatives to barley and enhancing beer quality for beer production.

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