Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Marius Loktu

Evaluation and improvement of shelf stability of New England IPAs

- In terms of oxidative degradation

Master's thesis in Food Science, Technology and Sustainability
Supervisor: Eivind Almaas
Co-supervisor: Christian Schulz and Lene Waldenstrøm
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Abstract

Today, New England IPAs are typically offered with a short shelf life and with recommendations to be stored cold, as the style seems to be particularly prone to oxidation. The main goal of this thesis was to explore if any additives introduced during packaging could slow down the oxidation of a New England IPA and positively affect its shelf stability.

A New England IPA was produced and packaged with five different additives pre-dosed into the cans. Seven different versions (samples) were made. One sample was untreated and canned as is, and one was intentionally oxidized (not pre-purged with CO_2 and low filled). The remaining five had the following additives added to the cans: Sulfur dioxide, sulfur dioxide and ascorbic acid, tannin blend 1 (EnartisTan SLI), tannin blend 2 (Enartis Hideki®) and inactivated yeast (Lallemand Pure-Lees Longevity™). The canned samples were stored at ambient temperature to emulate realistic storage conditions and accelerate oxidation. The samples were assessed over time (day 1, day 30, day 92 and day 126) by conducting sensory analysis (Napping® with UFP) to see if any sensory differences could be perceived between the samples. Including supporting chemical analyses to see if any chemical differences (changes in color with UV/Vis spectroscopy and observing patterns of compositional similarities and differences with NMR spectroscopy) could be observed between the samples.

The results from the sensory analysis indicated that all samples with additives added were able to slow down oxidation, as they kept better than the one without additives. But the result was considered preliminary, as the storage time needed to be longer to evaluate the total effect of the additives and because of the inactivated yeast being omitted from the sensory analysis due to a source of error. Of the additives examined, enological tannins had the most effect, especially those derived from oak (EnartisTan SLI). The results from the supporting chemical analyses were inconclusive. UV/Vis spectroscopy was only able to demonstrate that darkening of color occurred in the intentionally oxidized sample, while NMR spectroscopy provided limited insight and could not discriminate on utilized level if single samples differed more or less than others. However, patterns were observed between samples in general, as their differences increased the longer they were stored.

Sammendrag

I dagens marked selges som regel New England IPA med kort holdbarhet og anbefalinger om kald oppbevaring, da stilen tilsynelatende lett påvirkes av oksidasjon. Hovedmålet med denne oppgaven var å undersøke om enkelte tilsetninger tilsatt i emballeringssteget til produktet hadde evnen til å hemme oksidasjon i en New England IPA og påvirke holdbarheten positivt.

En New England IPA ble produsert og tilsatt fem ulike tilsetninger direkte i boksene før tapping. Sju forskjellige versjoner (prøver) ble laget. Én av prøvene ble ikke tilsatt noe og bokset som den var, mens en annen prøve ble oksidert med vilje (ikke purget med CO_2 og lav fyllhøyde). De fem resterende prøvene ble henholdsvis tilsatt: Svoveldioksid, svoveldioksid og askorbinsyre, tanninblanding 1 (EnartisTan SLI), tanninblanding 2 (Enartis Hideki®) og inaktivert gjær (Lallemand Pure-Lees Longevity™). Prøvene ble deretter lagret i romtemperatur for å etterligne realistiske lagringsforhold og fremskynde oksidasjon. Prøvene ble vurdert over tid (dag 1, dag 30, dag 92 og dag 126) og analysert sensorisk (Napping® med UFP) for å avdekke om eventuelle sensoriske forskjeller mellom prøvene kunne oppdages. Samt kjemiske analyser for å se om det kunne observeres noen forskjeller rent kjemisk mellom prøvene (forandringer i farge vha. UV/Vis spektroskopi og observasjon av komposisjonelle likheter og ulikheter i form av mønstre eller trender vha. NMR spektroskopi).

Resultatene fra den sensoriske analysen antydet at alle prøvene med tilsetninger hadde evnen til å bremse oksidasjon, da de holdt seg bedre enn prøven uten tilsetninger. Men resultatet ble sett på som innledende, da lagringstiden av prøvene må være av en lenger art for å evaluere den totale effekten av tilsetningene, og fordi prøven tilsatt inaktivert gjær ble utelatt fra den sensoriske analysen grunnet en feilkilde. Av tilsetningene vurdert, hadde de enologiske tanninene mest effekt, og da spesielt de tanninene som stammet fra eik (EnartisTan SLI). De støttende kjemiske analysene gav ikke nevneverdige resultater. UV/Vis-spektroskopi viste kun at prøven som var oksidert med vilje utviklet en mørkere farge. Ut ifra nivået datamaterialet fra NMR-spektroskopi ble vurdert, kunne det ikke observeres om enkeltprøver skilte seg mer eller mindre fra hverandre. Men det ble observert mønstre som tydet på at forskjellene mellom prøvene generelt økte jo lenger de ble lagret.

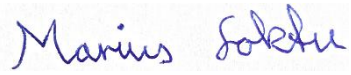
Acknowledgments

This master's thesis covers 45 ECTS points and is the final part of my Master of Science in Food Science, Technology and Sustainability at the Norwegian University of Science and Technology (NTNU) in Trondheim. It was carried out in collaboration with the Department of Biotechnology and Food Science, E.C. Dahls Brewery and Monkey Brew, from autumn 2021 to spring 2022.

First off, I would like to extend my gratitude for being able to shape my own project on a subject that really intrigues me, and that I think can benefit the brewing industry. As well as me directly, as I've opened my own brewery while attending the master's program part-time. Thank you to my main supervisor, Professor Eivind Almaas, who believed in the project and made me feel very welcome in the Almaas Lab research group, as well as accommodating me juggling two things simultaneously. I would also like to thank my co-supervisors: Postdoctoral researcher Christian Schulz, who has been quite a bit involved in everything, especially NMR. All while taking care of his newborn at the same time. And Assistant professor Lene Waldenstrøm, for helping me dive into the exciting field of sensory analysis, while always being accommodating and available to help. I would also like to thank Gaston Courtrade for staying late as the sample preparation for NMR dragged out. As well as everyone that contributed from the Almaas Lab Group by offering feedback.

Outside of NTNU, I would like to thank the sensory panel at E.C. Dahls, with a special mention to Nina Gregersen (lab engineer, E.C. Dahls) for bearing with me crashing her lab with all my samples. I would also like to thank the whole team at Monkey Brew, especially Pål Mikalsen Rolfsen, for letting me add weird stuff through their canning line.

Last, but most importantly, I would like to give a special thanks to my brother, Joakim Loktu, for the invaluable help offered. And my sweetest, Emilie Grimsrud, for the patience and loving support through a very work-intensive year.



Marius Loktu

Trondheim, June 5th, 2022

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1 Introduction

1.1 Craft beer, New England IPA, market and relevance

Even though craft beer and IPAs seem to be everywhere these days, it's humbling to think about that beer has been through many periods since brewing as a craft emerged more than 5000 years ago (Oliver, 2011). It's only 500 years since hops started being a normal part of beer, and only 150 years since brewers started using isolated yeast strains to ferment their beer (Tonsmeire, 2014, p. 1). And since then everything has just escalated.

In the 1800s breweries were all over Europe and America. Europe and America had a focus on producing lagers, while Britain focused on ales (Oliver, 2011). The American lager moved over time away from its European roots, gradually becoming its own beer category due to the increased usage of other starch-containing ingredients like rice and corn instead of malt (Oliver, 2011), as American breweries developed the technology to be able to make beer from any starch-containing material (Meussdoerffer, 2009). The American beer industry drifted even more away from the European as prohibition became general law of the whole America and prohibited the consumption of alcohol from 1920 to 1933 (Meussdoerffer, 2009). It decreased the number of American breweries from 1243 in 1916 to 31 breweries in 1933. Prohibition laws had already affected some states and Canada since 1917 (Meussdoerffer, 2009). This caused lasting changes in both how Americans perceived beer and how it was produced. The restrictions of this period resulted in a lot of innovations that made for a powerful and efficient brewing industry where demand and capital were more important preconditions for brewing than the availability of suitable grains (Meussdoerffer, 2009). Leaving Americans with mass-produced and heavily advertised beer without much flavor, compared with their European counterparts (Oliver, 2011). But as traveling between the continents got more normal, Americans started realizing what they had been missing.

The modern craft beer movement is often traced back to the United States in 1965, when Fritz Maytag bought Anchor Steam Beer Company (Jesús Callejo, Tesfaye, Carmen González, & Morata, 2020). He avoided its closure, creating a portfolio of new beers with a diversity of different styles and inspired others to follow in the years after (Rotunno, 2015). Including a very important product in 1975 called Liberty Ale, who many consider to be the first modern craft india pale ale (IPA) (Rotunno, 2015). At that point, IPA was a traditional English style, but this adaptation used American hops which was one of the changes that eventually made

it into a separate category of beer (Strong, 2021). The 70's and 80's saw an American modernization of many traditional European beer styles, followed by many years of experimentation and creativity, driven by American craft brewers which had honed their brewing skills as homebrewers in the search of better beer than what America was left with after prohibition (Oliver, 2011). This movement would ultimately lead to new beer styles and the breadth of products we have available right now. Today, Anchor Liberty Ale would be regarded more like an American Pale Ale in taste, as the style has evolved during the last 45 years (Strong, 2021).

Fast-forwarding to present time, hop-driven beers like pale ale (PA), india pale ale (IPA) and New England IPA (NEIPA) are some of the most popular beer styles and have been some of the biggest contributors to increase the growth of the modern craft beer market (Baiano, 2021). In 2017, they accounted for approximately 20,3% of the total amount of craft beer sold globally, while IPA alone accounted for 27% of the total European craft beer market in 2017 (Baiano, 2021). In Norway, 24,5% of all the beer sales at the state-owned liquor stores "Vinmonopolet" were IPAs in 2021. In total 1 021 086 liters of IPAs were sold through this sales channel and 73,5% was produced in Norway. The category for pale ales comes on top of that. (Vinmonopolet, 2022) AS Vinmonopolet doesn't state numbers for the sub-style NEIPA. Patrick Pelsholen (personal communication, May 2022), brand manager for one of Norway's leading beer importer and wholesaler, CASK AS, shared that in 2021, 50% of their beer sales were IPAs (including the sub-style NEIPA), while 14% of the total beer sales were NEIPAs.

1.2 Research objective

A big concern with New England IPAs is that the style seem to degrade so quickly in terms of oxidation (Janish, 2019, p. 246), causing an unappetizing darkening of the once bright color and drastic changes to the aroma and flavor (Janish, 2019, p. 237). Having a short shelf life, the style needs to be consumed fresh for the consumer to be able to enjoy all the beverage has to offer before it becomes a shadow of itself. Today, NEIPAs are offered commercially with a short drinking window, preferably kept in an unbroken cold chain, and by some breweries even just sold directly to the consumers to avoid being stored at ambient temperatures in retail stores (Patrick Pelsholen, personal communication, May 2022).

In addition to the measures above, brewers have taken productional steps to improve the shelf life of NEIPAs. They are introducing as little as possible of dissolved oxygen to the product throughout production and packaging (Janish, 2019, pp. 247, 268), as well as adjusting the beer's malt composition to achieve lower levels of certain trace metals in the beer that can work as catalysts for the radical activation and cause further oxidation of various compounds in the beverage (Janish, 2019, pp. 245-248).

Browsing through the shelves at AS Vinmonopolet, most NEIPAs both marked with canning date and best before date, have shelf lives between 4 to 6 months. When contacted, Patrick Pelsholen (personal communication, May 2022) states that most NEIPAs in the market have best before dates ranging between 4 to 8 months from packaging. For their own portfolio they consider 6 months to be the minimum, as Norway's state-owned retailer, AS Vinmonopolet, does not accept receiving beer with less than 60 days until expiration. He shares the difficulties of striking the balance between selling the beer style as fresh as possible (like the breweries want them represented), and having a long enough best before date to be able to move them throughout the value chain without wastage. He also states the importance of including both canning date and best before date for transparency, as every beer and brewery operates with different shelf life.

As one of the bestselling categories in the craft beer segment these days (Baiano, 2021; Vinmonopolet, 2022), this causes logistical and economic problems in the whole value chain. Research that could improve shelf life could affect producers, wholesalers and retailers financially, which would also benefit the end consumers.

As different measures already have been taken in the production of the style, it could be interesting to look at it in a different way, in terms of giving brewers another tool in the toolbox to what they are already doing. **Thus, the main goal of this thesis is to explore if any additives introduced during packaging can slow down the oxidation of the final product and positively affect its shelf stability**. To obtain this goal, and assess the effectiveness of the additives, a sensory analysis is conducted to see if any sensory differences can be perceived between the samples. Supporting chemical analyses are also conducted to see if there are any chemical differences that can be spotted between the samples and contribute to the evaluation of oxidation between the samples.

The results are especially relevant for Norwegian producers of the style, as NEIPAs must be sold through AS Vinmonopolet or bars/restaurants, as breweries are not allowed to sell beers above 4,7% alcohol by volume (abv) directly to the consumer. This puts another step or two in the value chain between the brewery and the end consumer, where storage conditions and time can be an issue. This adds importance to keeping the products well on the shelf.

1.3 Delimitation of the thesis

The production process of brewing beer is not discussed in depth in this thesis, due to the scope of the thesis. The focus is put on additives added to a single style of beer and how it affects shelf stability in terms of oxidation. Enough information in terms of background and methodology is included to allow for copying the work, while some processes that is considered to have more impact on the oxidative stability gets more focus.

As a lot of the additives used in the thesis is made for the wine industry, some literature based on wine is included. As they are not of common usage in the beer industry or -literature.

Specific oxidative changes of every single possible compound in beer won't be gone through in detail as a lot are still disputed and the analyses performed won't touch base on that level of detail. The most relevant compounds will be introduced, but the focus will be on limiting the formation and activity of reactive oxygen species (ROS), as levels of oxygen and certain trace metals in the production and packaging of beer reduces the shelf life of beer (Chrisfield, Hopfer, & Elias, 2020; De Francesco et al., 2020).

The category of India Pale Ale (IPA) consists of both an American-, English- and Belgian style, as well as many sub-categories of American IPA (Strong, 2021). Only American IPA and its sub-category New England IPA will be included.

2 Background

This chapter will address background information relevant for the thesis and the research objective. To get a theoretical understanding of the product in question, the product group India pale ale (IPA), and more importantly, it's sub-style New England IPA (NEIPA) are briefly described. Before the main topic, oxidation of beer, and oxidation of this specific style are introduced. Background on the different additives relevant for the thesis are described, in the form of sulfur dioxide, ascorbic acid, tannins and inactivated yeast. At last, sensory analysis, with a focus on the methodology Napping® with Ultra-flash profiling (UFP) and complimentary data analysis, are addressed. Followed by a briefly description of the analytical methods ultraviolet–visible spectroscopy (UV/Vis) and nuclear magnetic resonance (NMR) spectroscopy and corresponding data analysis for NMR data.

2.1 Beer

Beer is a complex aqueous liquid consisting of more than 3000 different compounds, which makes keeping the product stable and the quality high a never-ending challenge for breweries all over the world (Guido, 2016). Time will always cause chemical changes in beer and the shelf life can be affected in various ways (Guido, 2016). What causes the most concern are changes that causes changes to the appearance or flavor of the product.

2.1.1 India Pale Ale (IPA)

A modern American IPA is described by the current Beer Judge Certification Program (BJCP) guidelines for beer (Strong, 2021) to be "A decidedly hoppy and bitter, moderately strong, pale American ale. The balance is hop-forward, with a clean fermentation profile, dryish finish, and clean, supporting malt allowing a creative range of hop character to shine through". They are also clear in appearance, but a light haze is also allowed (Strong, 2021).

Between 2005-2010 it was very popular to brew highly resinous, citrusy, and bitter IPAs, pushing the international bitterness units (IBUs) higher and higher (Bernstein, 2016). While also focusing on brewing stronger and stronger examples in terms of alcohol levels (Bernstein, 2016). In other words, pushing the style to its limits. In innovating industries, there are often reactions to what's currently the norm, and some breweries from the New England region of the United States started making a version of American IPA that focused more on the aroma and flavor, and not the bitterness (Bernstein, 2016; Strong, 2021). The

style caught the attention of consumers and other breweries, and grew in popularity throughout the 2010's, and evolved gradually to have even less perceived bitterness than in its beginning (Strong, 2021).

2.1.2 New England IPA

From a production standpoint they started utilizing newly developed hop varieties that were very fruit-forward (like Citra, Galaxy and Mosaic) (Bernstein, 2016). They also started turning to utilizing the hops more towards the end of the brewing process (Bernstein, 2016). By turning the focus on tropical fruit forward hops added late in the production process, and especially utilizing more of the hops as dry hopping in the fermenter, the style ends up having the juicy characteristics it's so well known for (Strong, 2021). The style also moved towards a grist of grains that included less caramel malt (Strong, 2021), and more flaked grains, like oats and wheat than the typical traditional American IPA. This increased the beer's body and gave it a smooth mouthfeel (Bernstein, 2016; Strong, 2021). At that point, this wasn't considered to be a separate beer style, but just a variation and way of producing an IPA that eventually became more and more popular. Many of the producers from the New England region (Alchemist, Hill Farmstead etc.) that first produced these types of beers still just market them as IPAs.

The BJCP guidelines for beer (Strong, 2021) describes New England IPA or Hazy IPA as many call it, "an American IPA with intense fruit flavors and aromas, a soft body, smooth mouthfeel, and often opaque with substantial haze. Less perceived bitterness than traditional IPAs but always massively hop-forward".

2.2 Oxidation of beer

Flavor stability is one, if not the most, important quality parameter in beer production (De Francesco et al., 2020). Gresser (2009) defines beer flavor stability as the beer's ability to keep its characteristics unaltered from the time of filling to the time of consumption. Even if some consumers, or some styles of beer can be appreciated with some oxidation, it is desired to avoid this from happening for as long as possible, as most beers are intended to be consumed fresh (De Francesco et al., 2020). And there will occur changes in aroma and freshness of the beer when it begins to stall (Gresser, 2009).

Beer staling is a sequence of degradations, characterized by the oxidation of beer components to form a variety of new compounds, where carbonyl compounds are considered to be the most important in terms of affecting beer flavor (Gresser, 2009). Even though the oxidative degradation of beer during aging is connected to a lot of different chemical changes, (E)-2-nonenal is probably the compound that is most frequently associated with oxidation (De Francesco et al., 2020). It's described as giving a cardboard, or wet paper note to the beer, and is a degradation product of oxidized unsaturated fatty acids (De Schutter, Saison, Delvaux, Derdelinckx, & Delvaux, 2009).

But as drawn from the work of Janish (2019, pp. 254-255) in this paragraph: Oxidative changes can be so much more than that, and a product can be strongly affected by oxidation even though not being at the level of tasting papery. That's especially true for hop forward product categories. Typically, a sweeter, more malty and caramelly taste develops, while fruity and floral esters from the hops, which is intense when the beer is fresh, will be gradually weakened and, eventually, lost. This is not necessarily due to breakdown or changes in the concentration of the hop flavor compounds themselves, but caused by the competition with oxidative compounds that are gradually appearing and increasing in the beer, and are picked up from a sensory perspective at various threshold levels. A decrease in bitterness is also normal during aging.

On a more chemical level, everything from oxidation of unsaturated fatty acids and polyphenols, to Maillard reactions, to degradation of carotenoids and hop bitter acids, to the acetalization of aldehydes and hydrolysis of esters and glycosides will contribute to the shelf stability and flavor changes of beer (De Schutter et al., 2009). Cendrowski, Królak, and Kalisz (2021) and Barril, Rutledge, Scollary, and Clark (2016) highlight the relevance of oxidation of phenolic compounds in beer, like polyphenols, which turns them into hydrogen peroxide and quinones which are both oxidizing agents in beer. De Francesco et al. (2020) and Gresser (2009) on the other hand, highlight that some of the biggest reasons for beer staling flavors appearing during storage is due to carbonyl compounds created by the oxidation of unsaturated fatty acids, higher alcohols and iso-humolones.

Not only flavor is affected. An increase in color occurs during beer storage, both due to Maillard reactions, and the oxidation and degradation of polyphenols (Callemien & Collin, 2007). This correlates with the findings from Hodzic, Karahmetović, Saletovic, and Šestan

(2007) who demonstrated that when polyphenol content in a beer increased, as did the color. They related the occurrence to the same reasons as Callemien and Collin (2007). Gribkova et al. (2022) and Habschied, Lončarić, and Mastanjević (2020) also mentions the interaction between oxidation of phenolic compounds and color.

In terms of shelf life, the importance of low oxygen content in the final packaged beer has been demonstrated and mentioned a lot throughout the years (Guido, 2016). Chrisfield et al. (2020) and De Schutter et al. (2009) explains well why it's so important, and the fundamental cause is explained in the following paragraph: When beer is exposed to oxygen, and the oxygen dissolves into the liquid, it kicks off a variety of reactions, many mentioned above. But the ground state of oxygen, the non-reactive triplet oxygen that is present in the liquid, does itself not cause instant oxidative reactions in the beer. But activated and reactive forms of oxygen (singlet oxygen, hydrogen peroxide and hydroxyl radicals etc.) can quickly be created due to catalysts, like transition metals, being present in the beer. These activated forms of oxygen are called reactive oxygen species (ROS), as they are readily available to further react with different compounds in the beer and cause staling compounds. (Chrisfield et al., 2020; De Schutter et al., 2009)

De Schutter et al. (2009) express that there are so many variables related to oxidative reactions by ROS, that the focus should be to decrease the formation of radicals to start with (De Schutter et al., 2009). Chrisfield et al. (2020) emphasizes the importance of minimizing oxygen and transition metals in the beer, to reduce the formation of radicals and heighten shelf stability. Iron (Fe^{2+}), copper (Cu^{1+}) and manganese (Mn^{2+}) are transition metals known to affect product stability of beer, as they work as catalysts in the formation of ROS (Chrisfield et al., 2020; De Francesco et al., 2020; Gresser, 2009; Guido, 2016). Malt and hops are the main contributors of these trace metals present in beer (Chrisfield et al., 2020). Manganese is especially relevant for heavily dry hopped beers, as it has the tendency to stick around in the beer after fermentation, compared to iron and copper, where much are removed during the boiling of wort and fermentation (Janish, 2019, pp. 242-245). Dry-hops are the biggest contributor to manganese (some hop varieties more than others), but unmalted and flaked grains, together with malted wheat are also contributors (Janish, 2019, pp. 242-245). Chrisfield et al. (2020) found out that the amount of loss of thiols (highly aromatic compounds) in dry hopped beer correlated with their manganese content.

In terms of oxygen exposure, brewers want to keep this as low as possible throughout production and until the beer is packaged. For packaging, 100 ppb of oxygen is typical what big scale breweries try to stay below (De Francesco et al., 2020). De Schutter et al. (2009) states that 20-50 ppb is normal oxygen exposure in modern packaging lines, and Henney (2019) recommends to stay below 50 ppb of total package oxygen (TPO), while also stating the importance of capping on foam to displaces and reduce the oxygen in the headspace of the can.

2.2.1 Oxidation of New England IPAs

Why New England IPAs seem to be particularly prone to oxidation brings many questions to the table. It's a fairly new style and not much academic research has been conducted on the matter. A report (from undergraduate students at Worcester Polytechnic Institute) conducted on the effects on dissolved oxygen and shelf life of a specific NEIPA, concluded with a bare 7 week acceptable shelf life (Smith, Schaeffer, Bosco, & Pickett, 2019). On the shelves in Norway, they are usually ranging from having 4 to 8 months long best before dates (Patrick Pelsholen, personal communication, May 2022).

Heavily dry hopped beers, like NEIPAs, are prone to high levels of transition metals, as hops contain high concentrations which will leech into the beer and stay there (Chrisfield et al., 2020). The style also utilizes more flaked grains, like oats and wheat, than what's been normal before this style emerged (Strong, 2021). All of this have the potential to bring higher levels of manganese into the finished beer (Janish, 2019, pp. 242-245).

Some of the most influential commercial brewers of the style states that striving to keep dissolved oxygen low throughout production is the factor that by far has the most influence on the final result (Raspuzzy, 2020).

2.3 Additives

Beer itself consists of many compounds that works as antioxidants, like sulfites, polyphenols and melanoidins (De Francesco et al., 2020). The biggest share of polyphenols in beer originates from the malt, but some also originates from the hops (De Francesco et al., 2020). Some of these are able to react with free radicals and some are capable of chelating transition metal ions (De Francesco et al., 2020). The same type of compounds from

external sources could be added as additives to improve the shelf stability of beer. Guido (2016) mentions that additives either must be able to scavenge peroxides or trap metal ions to be effective in beer. As an example, the additive sulfur dioxide is capable of dealing with oxidizing agents like hydrogen peroxide and quinones in beer, which are byproducts from the oxidation of polyphenols (Barril et al., 2016; Cendrowski et al., 2021).

2.3.1 Sulfur dioxide (SO_2)

Sulfur dioxide (SO_2) is a colorless gas that is water soluble and already naturally present in beer as a component of yeast metabolism and fermentation, where the yeast reduces sulfate present in the grist or the water to sulfur dioxide (Guido, 2016). It has also been used as an additive in wine as an antioxidant for hundreds of years. In an aqueous solution, bisulfite- (HSO_3^-) and sulfite ions (SO_3^{2-}) will be formed, together with molecular SO_2, which are the three forms of free SO_2 (See Figure 1) that will present in the media (Waterhouse, Sacks, & Jeffery, 2016, pp. 140-142). These will all be present in different concentrations in the product depending on the pH of the medium, but will mainly consist of bisulfite (Waterhouse et al., 2016, pp. 140-142). The SO_2 will also be present in bound form with either stable or unstable compounds (see Figure 1), and the total of free and bound SO_2 makes up the total SO_2 in the media.

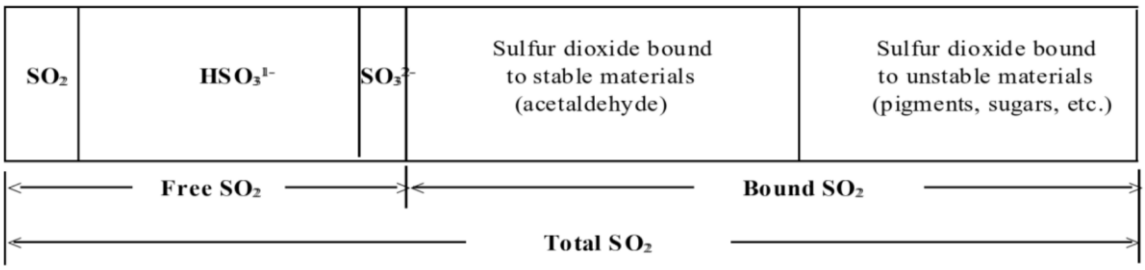

Figure 1 - A visual representation of the different forms of free SO_2: molecular SO_2, bisulfite- (HSO_3^-) and sulfite ions (SO_3^{2-}), and the SO_2 that is bound with compounds in the media. (Eisenman, n.d.)

As can be seen in Figure 2, sulfite has two different functions in terms of affecting shelf stability of beer. Firstly, by working as an antioxidant and delay the formation of radicals, thus preventing oxidation from occurring. Secondly, by forming adducts with carbonyl staling compounds and masking oxidative flavors that has already been created. For

example, the typical cardboard flavor that develops in very oxidized beer due to long chained and unsaturated aldehydes (Guido, 2016). The binding with free bisulfite ions makes these compounds non-volatile, giving them a significantly higher flavor threshold than the free carbonyl compounds (Guido, 2016). The antioxidative function of SO_2 isn't necessarily a direct interaction with oxygen, but the bisulfite ion inhibiting the Fenton reaction by reacting with hydrogen peroxide (H_2O_2) and thus preventing chemical oxidation from happening (Lisanti, Blaiotta, Nioi, & Moio, 2019). Pons-Mercadé et al. (2021) emphasizes that SO_2 has a slow reaction directly with oxygen, but that it's so widely used due to its ability to react and deal with hydrogen peroxide.

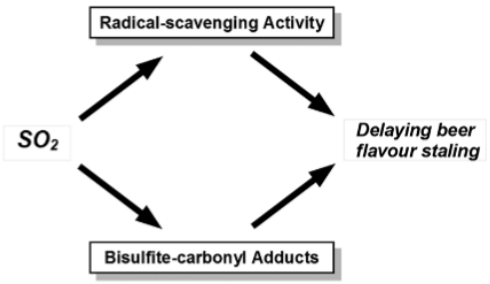

Figure 2 – Sulfite's two modes of action to help with flavor staling in beer (Guido, 2016).

When using SO_2 to prevent oxidation in beverage production it's normal to use the additive potassium metabisulfite (E224) (Guido, 2016; Waterhouse et al., 2016, p. 146), which is a crystalline salt that contains 57,6% sulfur dioxide (Vinlab, 2017). The Australian Wine Research Institute (AWRI, n.d.-a) states that about 4 mg/L of SO_2 is needed to consume 1 mg/L of oxygen, while Sacks and Howe (2015); Waterhouse et al. (2016, p. 142) states that 20-40 mg/L of free SO_2 is needed to avoid oxidation of wine. But only about 35-40% of an addition is typically yielded as free SO_2 due to the occurrence of binding with compounds in the media (AWRI, n.d.-a). From a sensory aspect an addition should only be problematic when the molecular SO_2 is above 2 mg/L, which is a form of SO_2 that correlates with the pH of the product (Sacks & Howe, 2015; Waterhouse et al., 2016, p. 142). AWRI (n.d.-b) also emphasizes that it is the molecular form that can cause an off-odor. The amount of molecular SO_2 can be calculated by knowing the products pH and its amount of free SO_2 (Malfeito-Ferreira, 2019, p. 231) (See Appendix H, figure 18 for more information).

2.3.2 Ascorbic acid

Ascorbic acid has a very high oxygen consumption capacity, both in terms of speed and the total amount of oxygen it's able to consume (Pons-Mercadé et al., 2021). When comparing antioxidative additives utilized in wine production, Pons-Mercadé et al. (2021) found that that 100 mg/L of ascorbic acid consumed 4,2 times more oxygen than SO_2, and at rate that was 350 times faster. The consumption happened almost instantly.

Barril et al. (2016) describes the antioxidative function of ascorbic acid (E300) to be a quick reaction with molecular oxygen (catalyzed by present metal ions), producing hydrogen peroxide (H_2O_2) and dehydroascorbic acid (which both can be dealt with by sulfur dioxide). As ascorbic acid has a low redox potential, this reaction will happen instead of the normal phenolic oxidation described briefly in chapter 2.2. In other words, ascorbic acid can be added as a sacrificial compound for the phenolic compounds that would normally be targeted by the molecular oxygen, and force another chain of reaction to avoid phenolic breakdown. (Barril et al., 2016)

Using ascorbic acid without the addition of SO_2 at the same time will not help avoiding oxidation in the end, just changing the chain of reactions. SO_2 is needed to bind the hydrogen peroxide created, which otherwise will cause further oxidative reactions even though the oxygen is consumed. (Pons-Mercadé et al., 2021)

2.3.3 Tannins

Tannins are polyphenolic compounds that occur naturally in fruit and vegetables (Ugliano, Slaghenaufi, Picariello, & Olivieri, 2020), as natural protectants towards external threats (Enartis, 2021). Ugliano et al. (2020) states they are of big importance in winemaking, both in terms of stability of color, aroma and structure. Wine has a natural source of tannins from the skins and seeds of the wine grapes, as well as from oak aging which is another source of tannins (Ugliano et al., 2020). In beer, tannins are present in the husk of the grains or in the hops utilized, and they lend some balance in terms of astringency to the product (Jakob, 2011). They also react with proteins and peptides in the beer to cause a stable haze (Jakob, 2011).

In modern winemaking it is normal to add different commercial preparations of enological tannins to either address deficiencies in the raw material, to obtain different organoleptic characteristics, to aid in clearing or to increase the antioxidant capacity and make the

product more robust in terms of oxidation (Ugliano et al., 2020). Many of the effects of tannins in nature also works after they've been extracted from their natural habitat, making them fitting utilized as additives (Enartis, 2021). Tannins have a multi-effect, being able to chelate and precipitate problematic transition metals out of solution, while also being able to react directly with free radicals produced or present in the media (Enartis, 2021). Both making the product less prone to oxidation.

Chemically, tannins belong in a group of compounds that is called polyphenols and can be split into two main groups: Condensed and hydrolysable tannins (Arapitsas, 2012). Condensed tannins are proanthocyanidins which derives from catechin and tara tannins, together with gallic acid- and quinic acid esters (Arapitsas, 2012). Hydrolysable tannins consist of multiple esters of gallic acid, together with glucose and derivates of their oxidative products (Arapitsas, 2012). Hydrolysable tannins are often further differentiated by gallotannins and ellagitannins (Landete, 2011). Oak is especially rich in ellagitannins, which are able to reduce oxidation-derived quinones (Ugliano et al., 2020).

Ugliano et al. (2020) states that classifying tannins after their antioxidant capacity is challenging due to the complexion of tannin composition and that assays for antioxidants often produce contradictory results. But found out in their own research that oak-derived tannins were able to quickly consume oxygen in wine and had the highest oxygen consumption of those tested. This is consistent with the statement from oenological producer Enartis regarding their EnartisTan SLI tannin product made from untoasted American oak, having extraordinary capabilities of scavenging oxygen and radicals (Enartis, 2019, p. 57). They also state that it has the ability to lower the redox potential of the product slightly and chelate out metals, which can increase the shelf life of the product (Enartis, 2020b).

Enartis (2021) has another product called Hideki®, made as a blend of tannins of different composition and chemical structures. Consisting of molecular fractions of both gallic, ellagic and condensed tannins, chosen for their antioxidant and microbiostatic efficiencies (Enartis, 2021). Condensed tannins showed protective effect on flavor stability and color preservation when added to beer in the study by De Francesco et al. (2020). More detailed, it revealed antioxidative activity in the form of scavenging oxygen, being able to inhibit free radicals and chelate with Fe^{3+} (De Francesco et al., 2020).

2.3.4 Inactivated yeast

It has for long been known that aging wine on the dead yeast cells from its fermentation process can contribute flavors and mouthfeel to the final product, due to the release of polysaccharides and mannoproteins (Pons-Mercadé et al., 2021). But there has also been reports that this production method ends up giving more stable products in terms of less oxidation and longer shelf life, although the mechanism behind it being unknown (Pons-Mercadé et al., 2021). The release of glutathione by the yeast has been brought up as a possible reason for their ability to consume and scavenge oxygen. (Pons-Mercadé et al., 2021)

Bahut et al. (2020) showed that the amount of glutathione present in the inactivated yeast products didn't necessarily correlate with the grade of oxidative stability offered. But proposed based on their findings, that other compounds that co-accumulate in the process of enriching the yeast derivatives with glutathione, counts more towards the antioxidant effect, than glutathione itself. Pons-Mercadé et al. (2021) also concluded that there must be other mechanisms involved than just the glutathione release after conducting their own research. Their research indicated that the level of glutathione released from the inactivated yeast affected the level of oxygen consumption, but that it didn't justify the whole consumption.

Companies like Lallemand, who produces yeast and other products for the beverage industry, produces different inactivated yeast additives. Lallemand (2016) states their protective effect towards oxidation should give the product an improved aromatic freshness, intensity, and color. But the additives can also add to the mouthfeel of the product due to its polysaccharide content and the phenomenon with aging on yeast mentioned above (Lallemand, 2016).

Lallemand (2021) has an inactivated yeast additive called Pure-Lees™ Longevity, which is developed to protect wine against oxidation during aging, due to its capacity to consume high levels of dissolved oxygen. It is an inactivated yeast additive where the speed of oxygen consumption and its total consumption capacity is optimized (Lallemand, 2021). An addition of 200 mg/L can scavenge 1 mg/L (1000 ppb) of dissolved oxygen (Lallemand, 2016). And their own research shows Pure-Lees™ Longevity (400 mg/L) being more efficient to protect color and aromas (in terms of thiols) in wine from oxidation, compared to SO_2 (60 mg/L)

(Lallemand, 2016). A recent study by Pons-Mercadé et al. (2021) supports this efficiency of inactivated yeast additives, and specifically the product Pure-Lees™ Longevity, which is included in the study where they compare common wine antioxidants with inactivated yeast additives in terms of oxygen consumption rate and total consumption capacity. At levels of 30 mg/L of SO_2 and 400 mg/L Pure-Lees™ Longevity, the inactivated yeast were able to consume 1,2 times more oxygen than the SO_2, and at a rate that was over 3 times higher. Another inactivated yeast product performed even better. (Pons-Mercadé et al., 2021)

The company itself, Lallemand (2016), did not observe any yeast flavor or off-flavor from sensory analysis trials performed with an addition of 400 mg/L. They also state that the product maintains up to 9 months of scavenging effect, or until the additive's maximum oxygen consumption capacity is reached (Lallemand, 2016). In other words when it's saturated with oxygen. Edouard Lordat (product manager SYD, Lallemand inc., personal communication, April 2022) informs that Pure-Lees™ Longevity works by adsorbing the dissolved oxygen through the sterols in the membrane of the inactivated yeast. And that the removal is irreversible, so that with long contact times, the cell walls won't resuspend oxygen back into the media (Lallemand, 2016).

2.4 Sensory analysis

Sensory analysis is a tool for quality control, research, and product development, and it provides a great range of data which can bring insight alone or in combination with chemical analyses (Rødbotten, 2015). In short, the human senses are used to evaluate sensory impulses, consciously or unconsciously, by detecting, measuring, analyzing, and interpreting these impulses within a framework of a methodology (Rødbotten, 2015). These impulses can be gathered by taste, smell, vision, hearing, physical touch or a combination, depending on what the goal is. (Rødbotten, 2015)

To get sensory descriptions of products, descriptive methodology is used (Lawless & Heymann, 2010). It's extensively used in sensory analysis, as it allows for gathering reproducible results of high level of detail and precision (Moss & McSweeney, 2022; Varela & Ares, 2012). Quantitative descriptive analysis (QDA) is the descriptive method that is traditionally utilized, and it has the ability to provide a complete sensory description of a

product, or narrowing the focus on selected attributes (Lawless & Heymann, 2010). The main disadvantage of both QDA and other traditional descriptive methods are that a trained panel must be utilized, which includes extensive training and calibration (Varela & Ares, 2012). This could make these methodologies problematic to use for research that either has a short timeframe, low resources, or limited time usage of the panelists, as it is very time-consuming and expensive to perform (Moss & McSweeney, 2022; Varela & Ares, 2012).

That is one of the reasons new rapid methods of sensory analysis have been developed. As they give flexibility on both time usage and requirements of panel training (Moss & McSweeney, 2022). In the last decades, multiple descriptive rapid methods have been developed, as described in the article: *Quick and dirty but still pretty good: a review of new descriptive methods in food science,* by Valentin, Chollet, Lelièvre, and Abdi (2012). One of these, called Napping® with Ultra-flash profiling (UFP), is often utilized to get descriptions of food and beverages (István & Kókai, 2021; Kemp, Pickering, Willwerth, & Inglis, 2018; Perrin et al., 2008; Pickup, Bremer, & Peng, 2018; Sereni, Phan, Osborne, & Tomasino, 2020).

2.4.1 Napping® with UFP

Napping® is a rapid sensory method, which provides a holistic evaluation by looking at the samples at a broader level of detail, than traditional descriptive methodology like QDA (István & Kókai, 2021). The protocol and approach are easier to learn and quicker to perform, which makes it a suitable method for untrained assessors, even consumer panels (István & Kókai, 2021). It is a fairly modern methodology, as it was first created in 2003 (Pagès, 2005), and published in 2005 (Pagès, 2005). The basis of the original method it's based on (projective mapping) is to position samples physically in a two-dimensional space, like on a big piece of paper, to get a representation of the relationship between the samples (Moss & McSweeney, 2022). The closer they are placed, the more similar they are perceived. And the further away they are placed from each other, the more different they are perceived (István & Kókai, 2021; Moss & McSweeney, 2022). Today it's more normal to use a digital sensory space on a computer screen. It's made to look like a tablecloth, hence the name Napping®, which originates from the French word for tablecloth, "nappe" (István & Kókai, 2021). The differences between projective mapping and Napping® are a bit unclear and some distinguish between the two and some don't (Hopfer & Heymann, 2013). But Napping® can be separated from projective mapping, as data collection for Napping® has to

be conducted in a rectangular space (Pickup et al., 2018), and the data gathered has to be analyzed with Multiple Factor Analysis (MFA) (Moss & McSweeney, 2022; Pickup et al., 2018).

The sample positions in Napping® and Projective Mapping does not provide any product descriptions and needs to be paired with another step to get descriptors (István & Kókai, 2021). Ultra-flash profiling (UFP) is the most normal complementary step that is used to achieve this (István & Kókai, 2021). After positioning the products, the panelists adds descriptors next to single samples, or groups of samples (István & Kókai, 2021). If the panelists doesn't utilize the same terminology to describe the same sensory property, it can be helpful to include a list of descriptors to make sure there is a collective understanding and agreement of the sensory properties in question across all panelists (Moss & McSweeney, 2022). Perrin et al. (2008) state the use of the same terminology yields better results and supports the use of a list of descriptors. But both Perrin et al. (2008) and Moss and McSweeney (2022) emphasizes the importance of still allowing and encouraging the panelists to use their own descriptors, even if not in the list, to maintain the free and spontaneous part of the method. Even so, if the panelists do not have the same terminology, the researcher needs to interpret each descriptor (Moss & McSweeney, 2022).

When Napping® is paired with a descriptive step that can explain the sample positioning provided by Napping®, it will provide a sensory overview of the samples that can be quite similar to more complex and conventional descriptive methods (Lê, Lê, & Cadoret, 2015). Pairing Napping® and ultra-flash profiling (UFP) will get you a similar representation and the main characteristics, but not necessarily the same level of detail and accuracy that the quantitative data from a conventional method will give you (Perrin et al., 2008). Moss and McSweeney (2022) mentions that the product space from the sample positioning can be quite similar compared with using a trained panel, but that a trained panel better will manage to identify small differences between samples (Varela & Ares, 2012). István and Kókai (2021) highlights the importance of panel training, both in terms of methodology, but even more importantly training on the product group in question and getting some sensory familiarity of it. As their results showed it helped to get more reliable and reproducible results when utilizing rapid methodologies.

As Napping® is not an ISO-standardized method and still is in development, some modifications that makes it more suiting for the products in question or the objective of the analysis, are accepted (Varela & Ares, 2012).

2.4.2 Data analysis of Napping® with UFP

Multiple Factor Analysis (MFA) is the most common data analysis used for Napping® data (István & Kókai, 2021; Moss & McSweeney, 2022). The Napping® data consists of X and Y coordinates of the placed samples from each panelist in the two-dimensional space (Perrin et al., 2008). MFA is a statistical method which groups variables into different blocks. It is a two-step method where Principal Component Analysis (PCA) is used to normalize the values of each block and then place them in a matrix before a new PCA is ran on the entire thing (Abdi & Valentin, 2007; István & Kókai, 2021) before the analyzed data is compared for similarities and differences with the original data set (Abdi & Valentin, 2007; István & Kókai, 2021; Kemp et al., 2018).

More practically, this statistical method takes into account each assessors' individual assessment of the sample, and places the products far away from each other graphically if they are globally perceived as different by the panelists (Perrin et al., 2008). Each individual evaluation is being equally taken into account for the global placement (Perrin et al., 2008), as MFA takes every individual difference into account, instead of averaging the data as in PCA (Varela & Ares, 2012).

Data analysis of word-count methods like ultra-flash profiling (UFP) are often performed with Correspondence Analysis (CA), as it is a well suited method of analyzing lexical tables, which are frequency tables with products and words (Kostov, Bécue-Bertaut, & Husson, 2014). The studies from Iobbi and Tomasino (2021), Sereni et al. (2020) and Bertaut and Pagès (2009) utilize CA for data analysis of UFP-data.

2.5 Chemical analyses

2.5.1 UV/Vis spectroscopy

UV-visible spectroscopy is a quick and flexible analytical method with a wide area of usage (Rocha, Gomes, Lunardi, Kaliaguine, & Patience, 2018). It measures the absorbance of light that is able to pass through a medium as a function of the wavelength (Rocha et al., 2018).

More practically it works by having a light source on one side of the sample, and a detector on the opposite side, which records the UV-visible light that is transmitted through the media at a specific wavelength (Rocha et al., 2018).

Beer color can be determined quantitatively based on light absorbance with the standardized color unit EBC (European Brewery Convention), which is a method where the beer samples is measured in a cuvette in a spectrometer at a wavelength of 430 nm (Koren et al., 2020; Villa, 2011). To get an accurate determination of color, the beer sample must be free of turbidity and hence filtered (Koren et al., 2020; Villa, 2011). A wavelength of 430 nm was standardized as it showed the most variation for pale beers (Koren et al., 2020), and corresponds best with what is visually obtained by the human eye (Anger, Schildbach, Harms, & Pankoke, 2009).

EBC color = A_{430} *x 25 x Dilution Factor* with the usage of a 1 cm cuvette (Villa, 2011).

2.5.2 NMR spectroscopy

^{1}H nuclear magnetic resonance (NMR) spectroscopy is a non-targeted analytical technique where signals are produced for all chemical species that contains protons (Defernez & Colquhoun, 2003). ^{1}H nuclei are stable isotopes of hydrogen, called Protium, where the nucleus contains only one proton (Fjellvåg, 2021). The analysis provides information on the local magnetic fields around these atomic nuclei, and thus providing comprehensive information of chemical structures (Tampieri, Szabó, Medina, & Gulyás, 2021). The basic principle of NMR spectroscopy are molecules interacting with certain radio frequencies (Riswanto et al., 2022), as hydrogen nuclei behaves as small magnets (Clark, 2019). By immersing a sample in a strong magnetic field, the spin properties of these atomic nuclei are affected (Tampieri et al., 2021). When the proton flips from one magnetic alignment to another caused by the energy of the magnetic field (Clark, 2019), the resonance frequencies of the sample can be detected (Tampieri et al., 2021). This will appear as different peaks on a spectrum of frequencies (Tampieri et al., 2021), a proton spectrum (often called NMR spectrum) (Clark, 2019). Where the peaks appear depends on what the hydrogen atom is attached to within a molecule, as this will affect the strength of the magnetic field needed to bring the hydrogen into resonance (Clark, 2019). The resonance frequency of a sample is

also dependent on the chemical shift of the proton (Hoffman, 2006). Common practice is to measure the chemical shift in relevance to the solvent peak in the proton spectrum against an internal reference signal, like the reference Trimethylsilylpropanoic acid (TSP) in the solvent deuterium oxide (D_2O) (Hoffman, 2006).

The intensity of the resonance frequencies can also be measured (Tampieri et al., 2021), as the sizes of the peaks gives information regarding the numbers of hydrogen atoms reacting at that frequency (Clark, 2019). The sample is spun to avoid any variations from the magnetic field or the NMR sample tube (Tampieri et al., 2021).

2.5.3 Data analysis of NMR spectroscopy

NMR is extensively used in the analysis of food products (Riswanto et al., 2022), and the analysis is able to create an NMR spectrum containing a broad range of compounds (Defernez & Colquhoun, 2003). So broad that it can be hard to know what to look for. NMR fingerprinting is a suitable approach to deal with complex- and large sets of spectra, an approach looking at compositional similarities and differences between the samples from a broader perspective (Defernez & Colquhoun, 2003). By overlaying the spectra gathered for different samples from NMR, it can be hard to visually distinguish differences or similarities present, especially in large and complex data material (Defernez & Colquhoun, 2003).

Principal Component Analysis (PCA) is a statistical process, or multivariate ordination technique as stated by Syms (2008), and can be utilized to analyze and present these differences or similarities graphically in a plot, making them easier to evaluate. PCA reduces the dimensionality of large data sets, while still retaining most of the information and increasing interpretability of the data (Riswanto et al., 2022; Syms, 2008). It's an effective tool to explore relationships and trends between variables and find patterns in the data material, graphically displaying the dimensions that represents most of the variation in the data (Syms, 2008). Defernez and Colquhoun (2003) states that PCA is good at simplifying vast quantities of information contained in large sets of spectra, hence a suiting tool to explore the extensive information acquired through NMR-analysis.

3 Methodology

In this chapter the execution of the practical work in the thesis is described in detail. From the production of the beer, the addition of the different additives and packaging in cans. To sensory- and chemical analysis performed over time, which lays the foundation for the results in the thesis. A list of equipment, ingredients, additives and chemicals utilized for all the steps in this chapter is presented in Appendix A.

The chapter starts off with a flow chart of the experiment (see Figure 3), presented as the sequence of steps performed.

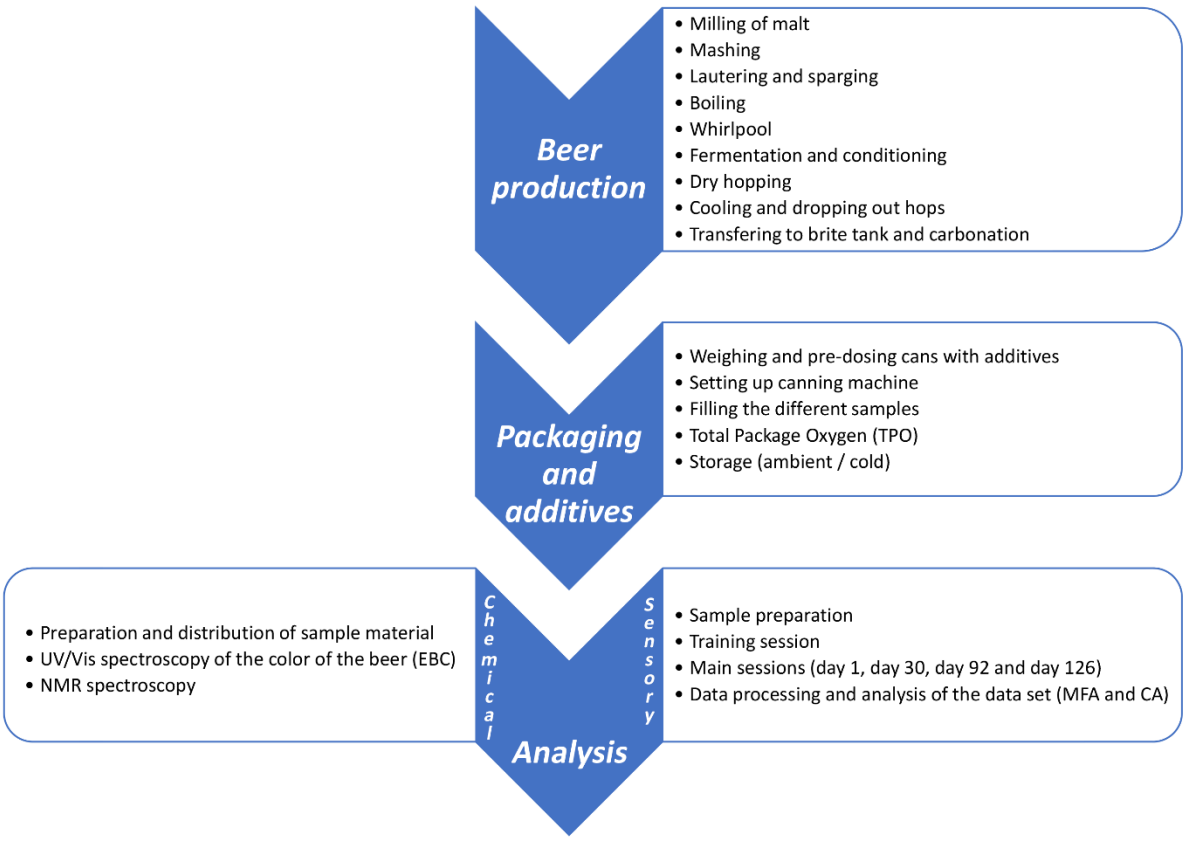

Figure 3 - Flow chart of the experiment presented as the sequence of steps

3.1 Production of beer

The production of the beer was carried out at the brewery, Monkey Brew, in Trondheim. The flowchart of the production of the beer can be seen in Figure 4, followed by a more detailed write up.

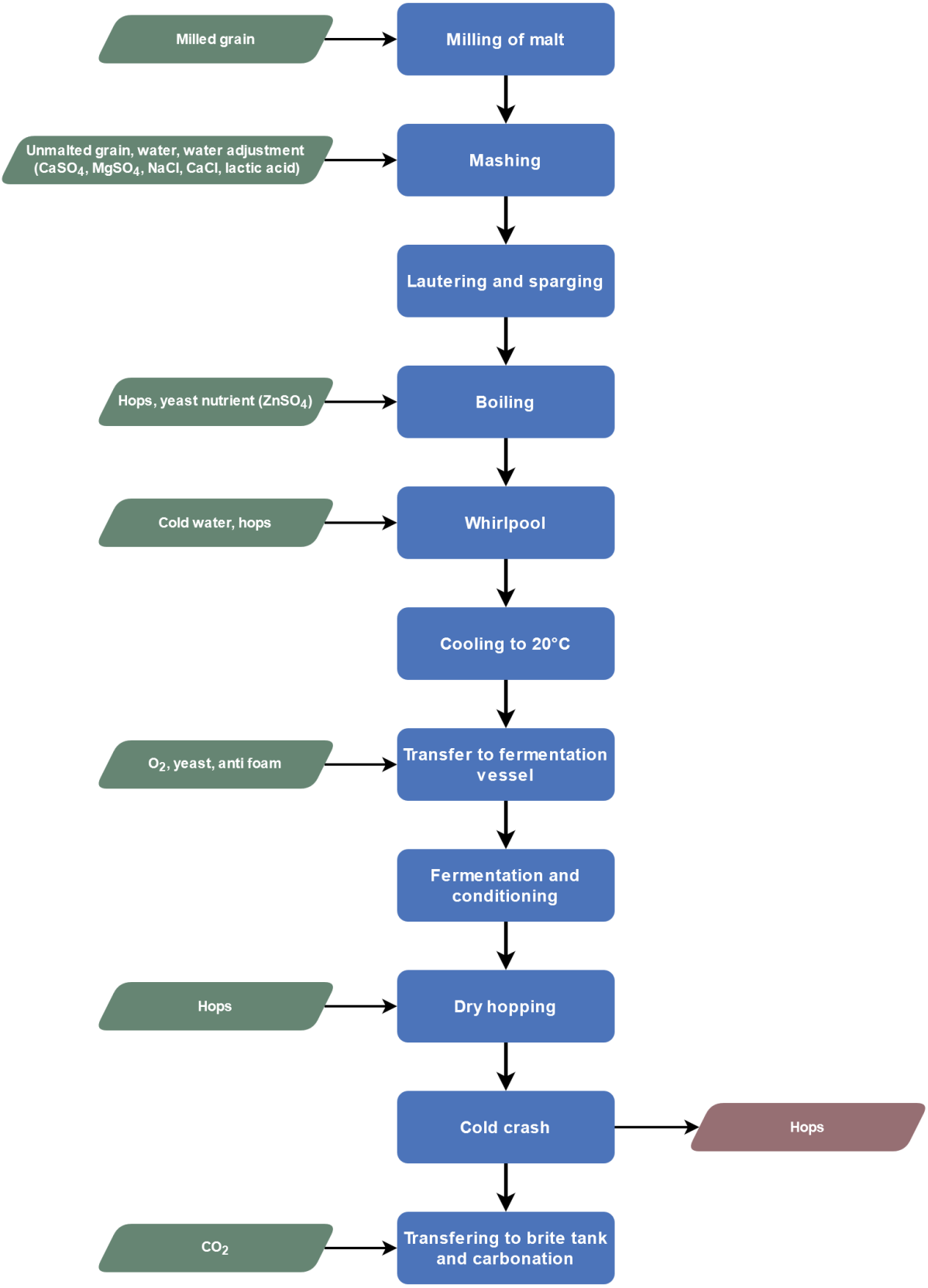

Figure 4 – Flowchart illustrating the productional steps of brewing the NEIPA for the experiment, including inputs and outputs in terms of ingredients.

The 632,5 kilos of malt and grains in the recipe (see Appendix B) were first milled through an electric malt mill, before being added to the mash tun together with 1825L of hot water to hit a mash-in temperature of 66,3°C and a mash thickness (water/malt ratio) just below 2,9 liters per kg. The water got adjusted with 430 g calcium sulfate ($CaSO_4$), 300 g magnesium sulfate ($MgSO_4$), 300 g sodium chloride (NaCl), 430 g calcium chloride (CaCl) and 1300 g lactic acid after Monkey Brew's recipe (see Appendix B).

After mashing for 80 minutes, the wort got pumped from below the lautering sieve, and to the boiling kettle. When drained, about 1100 liters sparge water that held 75°C was sprinkled above the grains, recirculated and pumped into the boiling kettle. 2200 liters of wort was collected in the boil kettle and boiled for 60 minutes. Hops *(co2-extract)* were added at the start of boil. *Tettnanger* pellet hops (2 kg) were added with 5 minutes left of the boil, together with 4 g zinc sulfate ($ZnSO_4$) as yeast nutrient. Additional 450 liters of cold water was added after the boil, and *Centennial* (3 kg), *Chinook* (2,5 kg) and *Mosaic* (2 kg) pellets were added to whirlpool at a temperature of 88°C. The whirlpool was conducted by circulation through a pump for 5 minutes, followed by a 15 minute long settling phase. Afterwards, approximately 2450L of wort got cooled to 20°C and transferred by pump to the fermentation vessel, adding pure oxygen through a diffusion stone during half the transfer time. Yeast (*Hazy Daze, third generation*) harvested from a previous batch were added directly to the tank through the hop-port at the top. *WHC Anti foam* (150 g) was added to the fermentation vessel to avoid foaming due to the high fill level.

After fermenting and conditioning at 20°C for 10 days, the beer was dry hopped with a total of 26 kilos of hop pellets (see brew sheet in Appendix C) for 5 days. Then cold crashed down to 2°C for 4 days, while hops being dumped from the bottom port daily. After the last dump, the beer got transferred to a pre-disinfected and pre-purged (45 minutes with CO_2 from the bottom valve) brite tank. The finished fermented beer was transferred by CO_2-pressure through clean and purged hoses, and carbonated to 2,6 vol/co_2 at 2°C through a carbonation stone in the tank for another three days before canning.

3.2 Addition of additives and packaging

The different additives got pre-dosed in marked cans the day ahead of canning (see Table 1). Weighed in with weighing boats on an analytical balance *(Mettler Toledo AB54-S),* that were calibrated with both its internal weight and an external calibration weight (50 g). 24 cans (440 ml) were marked and prepped with each addition, weighed in with a four decimal place accuracy. Cans marked with P01 and P02 were not filled with anything. Cans marked with P03 were filled with 0,0460 g potassium metabisulfite. Cans marked with P04 were filled with 0,0460 g potassium metabisulfite and 0,0220 g ascorbic acid. Cans marked with P05 were filled with 0,0220 g EnartisTan SLI. Cans marked with P06 were filled with 0,0220 g Enartis Hideki®. Cans marked with P07 were filled with 0,1760 g Lallemand Pure-Lees™ Longevity. All cans were stored in a marked cardboard tray, with another cardboard tray covering the top of the cans, awaiting to be filled with beer the next day.

Table 1 – List of samples and their respective treatment. Additives in grams per liter, and grams added per 440 ml can.

Sample	Variables	g/L	g / 440 ml can
P01	Beer without additions	-	-
P02	Intentionally oxidized	-	-
P03	Potassium metabisulfite (SO_2)	0,1040	0,0460
P04	Potassium metabisulfite (SO_2) + Ascorbic acid	0,1040 + 0,0500	0,0460 + 0,0220
P05	Tannin 1 (EnartisTan SLI)	0,0500	0,0220
P06	Tannin 2 (Enartis Hideki®)	0,0500	0,0220
P07	Inactivated yeast (Lallemand Pure-Lees™ Longevity)	0,4000	0,1760

The canning machine got sanitized and connected to the brite tank. It got driven in to operate smoothly before cans for this thesis were fed to the machine. The cans that were intended to be intentionally oxidized (P02) went through first. The purging-step of the cans with CO_2 was bypassed and the fill-level was decreased by approximately 40 ml, increasing the headspace in the can, and preventing the capping on foam. After dialing in the purging (3 seconds at 2 psi of CO_2) and fine-tuning the machine for correct fill- and appropriate foam levels, two cans were instantly taken out and brought quickly to E.C. Dahls Brewery and tested for Total Package Oxygen (TPO) using the instrument *Pentair Haffmans c-TPO* (following Haffmans' own protocol and operation instructions). Afterwards, 24 cans were removed and marked as P01. Then all the cans from version P03-P07, previously loaded with

the different additives, were fed into the machine, spread out in random order to decrease filling variables.

When all the cans were filled with beer and seamed by the canning machine, a weight control of the cans was performed. Right after, the cans were marked and put back into the cardboard trays. All the cans were gently shaken to make sure the additives were evenly mixed. The cans intended for analyses conducted at day 1 were brought to a refrigeration room at Dahls and kept at 3°C over the weekend to slow down any changes occurring, as the canning was carried out at a Friday. The rest of the cans were put in storage at Dahls, holding 20°C to emulate retail storage.

Some irregularities occurred when filling and seaming some of the cans pre-loaded with additives. This will be discussed in chapter 5.1.

3.3 Sensory analysis

The semi-trained tasting panel at E.C. Dahls brewery, in Trondheim, Norway, participated in the rapid sensory method, Napping® with UFP. The panel consisted of six employees (5 men and 1 woman), where everyone had been trained after the *"Carlsberg proficiency flavor identification standard"* (Nina Gregersen, lab engineer, E. C. Dahls, personal communication, April 2022). This includes principles of good practice for sensory assessment of beer, as well as both internal and external testing of basic flavor and beer faults. To be part of the panel, the panelists have to go through initial coursing and testing, as well as passing a yearly test on basic flavor. They also have to conduct at least 30 sensory assessments each year, and pass at least 4 external tests on beer faults. General information and principles regarding good sensory practice were not handed out to the participating assessors as it was deemed unnecessary.

The panel evaluated six different samples: The beer without additions (P01), the beer that was intentionally oxidized (P02), the beer with potassium metabisulfite added (P03), the beer with potassium metabisulfite and ascorbic acid added (P04), the beer with EnartisTan SLI added (P05), and the beer with Enartis Hideki® (P06) added. The six samples were served in all sessions (see Table 1). Sample P07 was excluded from the sensory analysis due to irregularities that occurred during packaging.

The sensory analysis was performed over five days in total. The main experiment of the sensory analysis was performed at four timeframes, spread out over a period of 126 days. With a training round conducted three days before this. The samples were stored at ambient temperatures of 20°C throughout the testing period, and samples needed for each round was put in cold storage the day ahead. The sample preparation and analysis were performed for the sample material at day 1, day 30, day 92 and day 126 after canning.

3.3.1 Preparations, serving and panel information

Each round was set-up in the software, EyeQuestion® *(version 4.11.61)*. Prior to the sensory analysis, the panelists were informed to exclusively evaluate flavor (taste and aroma) and how the samples should be placed and described. Appearance was not a subject for the sensory evaluation, as it was quantitatively measured with UV/Vis spectroscopy. A write-up was designed and handed out to the panelists in case they needed to revisit any of the information given (see Appendix D for write-up and Appendix E, Figure 13 for an example of sample placement in EyeQuestion®). A blank sheet of paper (A4) and a pencil were available for the panelists to sketch out the positioning and take notes before it was filled in digitally.

The sample order was balanced, with the plastic glasses being marked with randomly created 3-digit codes, unique for each session. The serving- and code order were also randomized. The glasses were presented to the panelists in a cluster. They had to follow the taste order presented in the software (randomized for every panelist), but they were allowed to revisit samples afterwards, for example to compare to samples against each other in terms of placement. The panelists had to bring their own laptop to conduct the sensory analysis digitally.

All the samples were served at 15°C in 300 ml transparent plastic glasses, including the water made available for every judge. They were encouraged to rinse their palate with water in between the samples. The sample size was 100 ml, and neutral biscuits (*Sætre Kaptein*) were available as palate cleansers. Contents from two cans of each sample (the same parallels used for the chemical analyses) were blended together in an Erlenmeyer flask, and marked with the three-digit codes used for that sample in that current session. Parafilm was put on top of the flasks to keep CO_2 in the product and oxygen out, until the panel got their samples served. Napping® with UFP was conducted by the panel with the evaluation of retro- and orthonasal odor and taste.

3.3.2 Training session

To familiarize the panel with the beer style and the methodology used, a training session was conducted in a meeting room at E. C. Dahls, three days before the first session of the main experiment, with five different commercial examples of the style, chosen and bought at AS Vinmonopolet. As well as a previous batch of the Monkey Brew beer being used for this project. All the samples were served blind, and the panel was unaware if they were served the same, or different beers, than the ones being served in the main experiment. In terms of what they were looking for, all they knew was that it was a shelf stability test. Being a panel trained in terms of beer and beer faults, they are aware that parameters linked to oxidized and unoxidized beer in general are relevant for storage tests. Prior to the session, the panel leader explained all panelists how to conduct the Napping® with UFP digitally, followed by a plenary discussion with questions from the panelists.

3.3.3 Main sessions (day 1-126)

The panelists conducted the first session (day 1) of the main experiment in a meeting room at E. C. Dahls, spread out around a big meeting table, not communicating with each other. For the last three sessions, everyone brought their samples (which where set-up in the same meeting room) to their own, closed offices out in the hall outside, due to the COVID-19 restrictions at that time.

Before the second session (day 30), a list of descriptors was created and introduced, to coordinate the terminology utilized by the panelists, and create a common agreement of the sensory properties (see Appendix F for the list of descriptors). The list of descriptors was created based on earlier descriptors utilized in the training session and first session, as well as through a dialogue with the panel. The panelists were not limited to any of these words, but it was expressed that if they wanted to describe something that coincided with the list, that they chose the terminology agreed upon in the list.

For the third session (day 92), the Napping® with UFP was conducted with one panelist missing due to a positive COVID-19 test, and for the last session (day 126), another assessor was served the samples 48 hours later than the others due to COVID-19 related quarantine (from different cans that were stored in ambient temperature for 1 day longer). Due to multiple postponements before this, and very uncertain times in terms of being able to gather the whole panel to execute the sessions, decisions were made to go ahead.

3.3.4 Statistical analysis

The results from the main sessions were analyzed using the statistical tool EyeOpenR® available in the EyeQuestion® software. The raw data was processed, and descriptors from UFP were interpreted where needed, before being imported back to EyeOpenR®. Multiple factor analysis (MFA) was used to analyze the Napping® data set (both the X and Y coordinates of the products placed on the map), while Correspondence analysis (CA) was used to analyze the frequency of the descriptors (UFP), with a filter on five words or more.

3.4 Chemical analyses

The initial preparation and distribution of the sample material pulled from all the cans for the chemical analyses were conducted before each session of sensory analysis, at the laboratory at E. C. Dahls. The sample material for the chemical analyses was harvested from the same two cans used for sensory analysis.

Two 15 ml centrifuge tubes of each sample were filled by pipetting beer straight out of the cans as soon as they were opened. They were filled to slightly foam over, so the cap could be placed on top of foam, to minimize oxygen exposure. The centrifuge tubes were held off for NMR-analysis until the storage time of 126 days had passed, and all the samples could be analyzed together. The centrifuge tubes were instantly placed in a freezer holding -20°C at E.C. Dahls after filling, moved frozen, insulated in newspaper and a polystyrene box, to an ultra-low temperature (ULT) freezer at Chemistry Block 3, NTNU, Gløshaugen, where the samples were kept at -80°C.

50 ml of each sample was poured gently into 250 ml Erlenmeyer flasks, and put on a platform shaker *(Heidolph Unimax 2010)* at 180 rpm for 15 minutes to degas the samples. These were used to determine beer color with UV/Vis spectroscopy at the laboratory at E. C. Dahls. The remaining content of the cans were used for sensory analysis as described.

PH-meter *(Radiometer Copenhagen PHM92 LAB and Hach Radiometer analytical Red Rod)* got calibrated with 4.01 and 7.00 pH buffer solution and decarbonated samples from the parallels of P01 at day 1 were ran.

3.4.1 Changes in color (EBC)

UV/Vis Spectroscopy *(Biochrom Libra S22)* was used to perform spectrophotometric analysis of the color of the beer (EBC). The beer samples were measured against distilled water as a control at an absorbance of 430 nm and a factor of 25, following the protocol for the EBC-method (see Chapter 2.5.1). The degassed beer samples were filtered through a sterile syringe membrane filter (*VWR 25 mm Syringe Filter w/ 0,45 µm Polyethersulfone membrane*) to reduce turbidity, straight into the glass cuvette (*HelmaAnalytics 10 mm*) used for every measurement. Each sample rested in the spectrophotometer for 30 seconds before being ran, for the sample to settle and stabilize after filtration. The samples were conducted in duplicates.

3.4.2 NMR spectroscopy

Sample preparation and the procedure for NMR acquisition were conducted by following the protocol "*Beer project NMR Manual*" developed and written by Adrian Antonsen, Leesa J Klau and Christian Schulz (Antonsen, 2021), and can be found in full in Appendix I. First the beer samples were removed from the freezer and defrosted for about 90 minutes. Each sample were gently shaken and 3 ml of each was filtered through a sterile syringe membrane filter *(0,2 µm, 25 mm diameter, luer lock)* into marked 5,0 ml Eppendorf tubes. The tubes were placed in an ultrasonic bath *(VWR Ultrasonic cleaner)* with their lids kept partially open for 10 minutes to degas the samples. Meanwhile, 1,5 ml Eppendorf tubes were marked and 80 µl of pre-made buffered stock solution *(500 mM sodium phosphate pH 7.4 buffer containing D_20 (99%) and TSP (1%))* were pipetted to each tube. Even though all the same samples are from the same beer, they were buffered to the same pH in case any of the additives affected them. Afterwards 750 µl of each sample was pipetted to each associated tube and the lid got closed.

After all samples were prepped, the tubes got vortexed one by one for four seconds, to mix and homogenize the sample and the buffer, before 600 µl of the mix was pipetted into a 5 mm NMR-glass tube *(Bruker LabScape™ Stream 5)*. The tube cap was attached, and the glass tube got wiped with lint free cleaning paper before getting placed at its given spot in the sample rack *(Bruker LabScape™)*. The sample rack was placed into the NMR's autosampler (*Bruker SampleJet)* and queued for NMR acquisition on a *Bruker AVANCE III HD 800 MHz NMR spectrometer* at the NMR Laboratory of Natural Sciences Faculty at NTNU.

The data processing of the spectra consisted of automated phase correction and baseline correction integrated in Topspin *(version 4.1.3)*, followed by calibrating the spectra manually by moving TSP peaks to 0 and defining peaks of interest. 114 peaks were selected, extracted, and integrated for all spectra. The TSP integral was set to 1 for all spectra as a reference. This work was conducted by Christian Schultz. The extracted data points include the peaks, as well as the data below the peak, as both together explains the intensity of the resonance signal, in other words how many protons causes said signal. The binned spectra data were exported as matrices in spreadsheets (.csv format) following the template from *MetaboAnalyst 5.0,* a web-based platform for metabolomics data analysis. The data was manually curated, as values below 0.0009 were formatted incorrectly by Excel and had to be manually formatted. The curated data were uploaded as spectral bins to the metaboanalyst.ca website, with no filtering applied and Pareto scaling utilized for data scaling. Multivariate tools of analysis accessible in the Metaboanalyst-platform were used for analysis and visualization of results.

4 Results and analysis

In this chapter results from the sensory- and chemical analyses will be presented and interpreted. The results cover baseline values like net volume of cans, Total Package Oxygen (TPO) and pH measured straight after packaging. Followed by results from Napping® with UFP, performed by the sensory panel at E.C. Dahls at different timeframes over a period of 126 days. Before looking at color changes as EBC-units (analyzed utilizing UV/Vis spectroscopy) and compositional similarities and differences between the samples (analyzed with NMR spectroscopy).

4.1 Baseline values of the NEIPA

Measurements of can volume and TPO were taken at the day of canning, while pH-readings were taken at day 1 of sample preparation. These are baseline values of secondary relevance for the total evaluation of the NEIPA.

The net volume of the cans utilized for the analyses differed between the different samples (see Table 2), as a consequence of the different additives added. P02 was intentionally low filled (417 ± 1,4 ml), while P05 (423 ± 4,4 ml) and especially P07 (346 ± 10,7 ml) were affected by the nature of the additives that were added (which will be explained in chapter 5.1). Respectively 11 and 7 cans of P05 and P07 made it through the canning line, from a pool of initially 24 cans per variant.

Table 2 - Net volume (ml) of cans used for the analyses reported as mean ± SD. The numbers are based on 8 cans for P01-P06, and 7 cans for P07. Calculation example can be seen in Appendix H, page 2.

P01	P02	P03	P04	P05	P06	P07
455 ± 0.5	417 ± 1.4	447 ± 0.8	449 ± 2.7	423 ± 4.4	452 ± 1.6	346 ± 10.7

The average TPO measured of the NEIPA was 132,7 ± 11,4 parts per billion (ppb) (see Table 3), and was based on three measurements of P01 performed 17, 22 and 28 minutes after canning. The oxygen in the headspace of the can (HSO) accounted for 47,3 ± 8,1 ppb, while the oxygen dissolved in the beer (DO) accounted for 85,3 ± 5,0 ppb. The baseline of oxygen in the packaged cans are quite a bit higher than desired if taking Henney (2019) advice on striving for a TPO below 50 ppb, or even following the 100 ppb that breweries typically try to stay below in general (De Francesco et al., 2020). One of the leading canning machine

suppliers (Wild Goose Filling) states the goal is to have DO as low as 10 ppb, but that keeping below 50 ppb is good practice (WildGooseFilling, 2020).

The amount of oxygen in the cans are higher than all the recommendations above, and the presence of a lot of precursors facilitates for the creation of reactive oxygen species (ROS) to further react with different compounds in the beer and cause staling compounds (Chrisfield et al., 2020; De Schutter et al., 2009). In other words, the starting point could have been better.

Table 3 - Measurements from Pentair Haffmans c-TPO of sample P01 taken at 17, 22 and 28 minutes after canning. Measurements presented as TPO, HSO, DO and headspace volume. n=3, results reported as mean ± SD.

Total Package Oxygen (TPO) - ppb	Headspace Oxygen (HSO) - ppb	Dissolved Oxygen (DO) - ppb	Headspace Volume - ml
132.7 ± 11.4	47.3 ± 8.1	85.3 ± 5.0	15.6 ± 0.7

pH-readings from both parallels of P01, performed at sample preparation at day 1, gave a pH of 4,47.

4.2 Sensory analysis

A rapid methodology like Napping® was chosen to be able to discern if there were differences between the six samples. With the addition of UFP, how, could also be explained, in terms of added descriptors to characterize the different products or groups of products. The results from the Napping® with UFP conducted by the semi-trained panel at E.C. Dahls, shows sample placement from the Napping® presented in two-dimensional MFA plots. Descriptors from the UFP is presented in CA plots, filtered on a frequency of 5 or more mentions per descriptor. The methodology is described in chapter 3.3.

The results presented in two-dimensional MFA plots gives a visual observation of the variations between the six samples evaluated. The closer the samples are, the more similar perception. The further away they are placed from each other, the more different they are perceived.

The results from day 30 and 126 are the ones that will be presented, as the goal is to observe the effect of the additives and if there are any changes to the samples over time. Day 92 showed the same tendencies as for those presented. Day 1 is not a realistic starting

point as a basis of comparison, as few commercial beers are available for the consumer that quickly after packaging, as well as not much oxidation is expected to occur by that time. It was however included as a baseline, and to explore if any of the additives would affect the sensory perception of the NEIPA themselves. The MFA- and CA- plots for day 1 and day 92 can be seen in Appendix G.

A list of descriptors was included for day 30 (see Appendix F), to make the assessors use the same terms and have a collective understanding and agreement of the sensory properties in question (Moss & McSweeney, 2022). Some of the panelists still chose to use quantitative adjectives like "slightly" before some of the descriptors, although they were instructed not to. Like Perrin et al. (2008) did in their study on wines when receiving quantitative adjectives in their data material, these adjectives were removed and the descriptor kept. The reason for doing so is the assumption that even a slight presence of a descriptor shows presence. Other adjustments that were performed on the data material from UFP for session 30, 92 and 126 were fixing obvious errors (punctation, obvious formatting mistakes etc.), changing the form of a word or similar words towards the ones listed in the list of descriptors (sweetness to sweet, citrus to citrusy, not fresh and old to oxidized etc.), and the removal of words that weren't really descriptive (good, undrinkable, unpleasant).

The Napping® results from day 30, as seen in Figure 5, shows that P01, P05 and P06 are fairly close to each other on the left side of the plot, indicating that the samples are perceived similar by the panel. While P03 and P04 are respectively placed on the lower and upper side of the plot, both towards the middle of the X-axis. Most of the explained variance is expressed by the first dimension, meaning these two NEIPAs are perceived somewhat similar and described to be sulfury (see Figure 6) by the panel (as dimension 2 only explains 18,47% of 85,76% explained variance in the data set). The NEIPA with the addition of both SO_2 and ascorbic acid (P04) is perceived sweeter than the NEIPA with the singular addition of SO_2 (P03). An off-flavor from the sulfur dioxide shouldn't theoretically be an issue with a beer at pH 4,47, as the added addition is far below 2 mg/L molecular SO_2 (see calculations in Appendix H), stated to be the sensory threshold value by Waterhouse et al. (2016, p. 142) and Sacks and Howe (2015). However, the addition of SO_2 and ascorbic acid apparently affected the sensory perception, as P04 was also perceived different to the

other samples at day 1 (see Appendix G, Figure 14). Although no descriptors from UFP were included by the assessors that would explain the difference in placement.

P02 is on the other hand placed far away on the x-axis, which explains 67,29% of the variance and indicates that the panel perceived it very different from P01, P05 and P06 placed on the opposite side of the plot. This correlates with the descriptors given from UFP (see Figure 6), where the descriptor “oxidized” is placed close to P02, while descriptors like “juicy”, “fruity” and “tropical” are placed around the cluster of P01, P05 and P06. Descriptors that is connected to how the style is supposed to taste fresh (Strong, 2021). The BJCP guidelines for beer (Strong, 2021) describes the term juicy as hops that has the same quality as fresh fruit juice, especially towards the tropical realm of fruits.

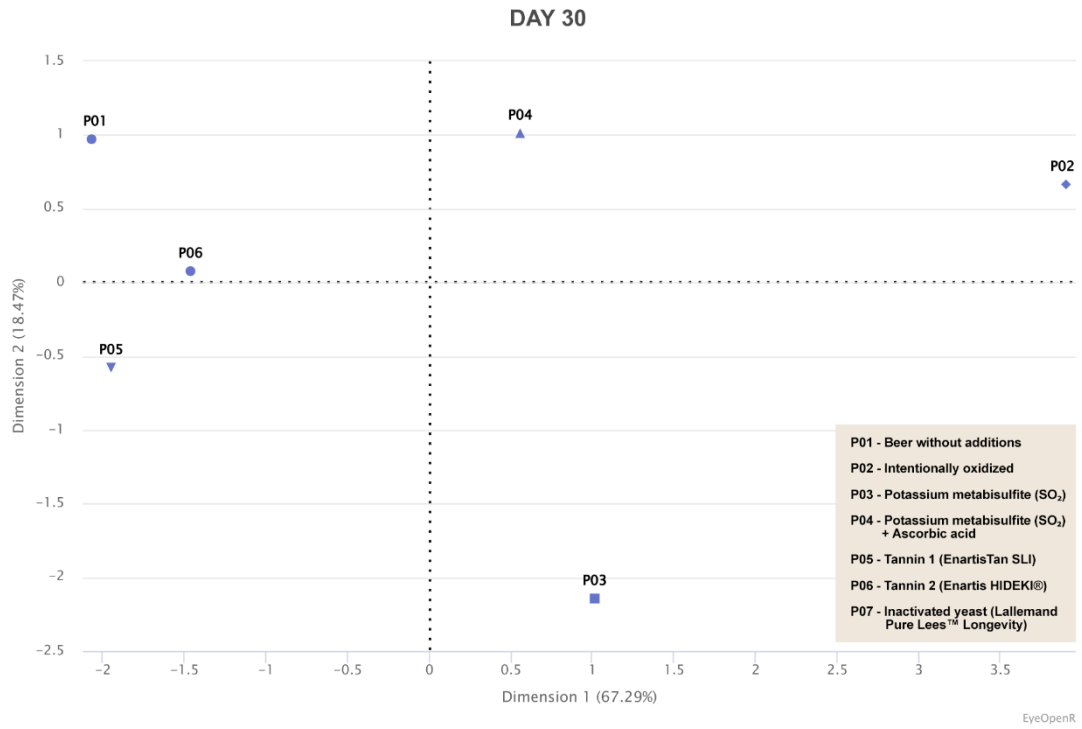

Figure 5 - MFA plot obtained from day 30 of Napping® with 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The X-axis (dimension 1) accounts for 67,29% of the explained variance, while the Y-axis (dimension 2) accounts for 18,47% of the explained variance. Together the plot accounts for 85,76% of the explained variance in the data set.

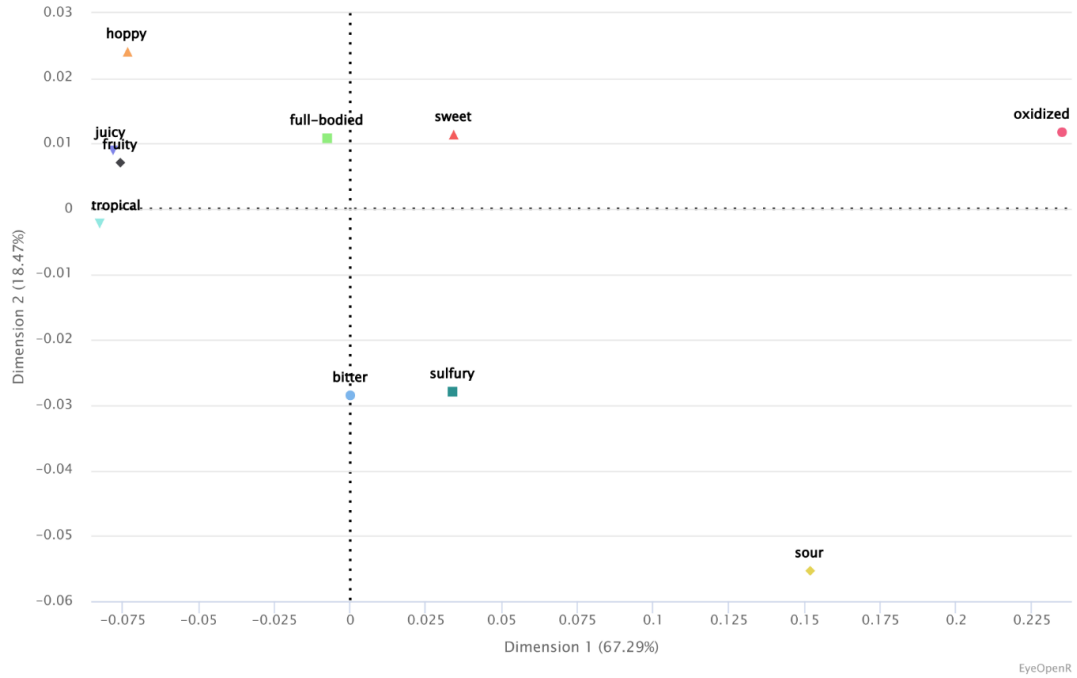

Figure 6 - Two-dimensional CA plot containing descriptors from UFP used by the panelist at a frequency of 5 or more at day 30 of Napping® of 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The X-axis (dimension 1) accounts for 67,29% of the explained variance, while the Y-axis (dimension 2) accounts for 18,47% of the explained variance. Together the plot accounts for 85,76% of the explained variance in the data set.

The Napping® results from day 126, as seen in Figure 7, shows that the panel still perceive the two samples with the addition of tannins (P05 and P06) to be very different from the intentionally oxidized sample (P02), being on opposite sides of the x-axis (which explains 48,18% of the variance). The sample without any additions (P01) has on the other hand moved towards P02 (see Figure 7), and the CA-plot in Figure 8 indicates that P01 is perceived less “tropical”, “hoppy”, “fruity” and “juicy” by the panel, than at day 30. Placement of sample P03 and P04 are pretty consistent relative to P02 throughout. This can indicate that the additives have an antioxidative effect, unlike sample P01 which seems to gradually pick up oxidative notes, both explained by the sample placement and the descriptors added. This correlates with Janish (2019, p. 255) writing about fruity esters from the hops gradually weakening, as well as oxidative compounds camouflaging hop flavor compounds, in hop forward beers. The descriptors that were connected to how the style is supposed to taste fresh, are still closely connected to P05 and P06, while the descriptor “oxidized” is still in close proximity to P02 (see Figure 8). The results from Napping® with UFP seem to go in the direction that P05 and P06 keeps the best, with P05 keeping slightly better of all the additives added.

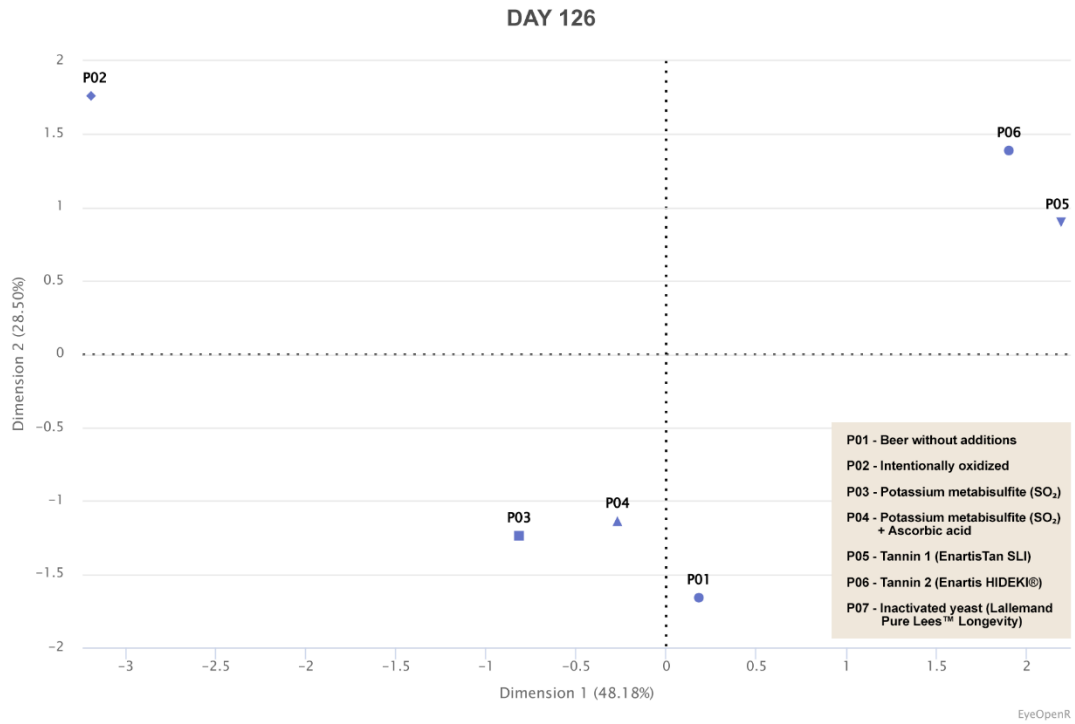

Figure 7 - MFA plot obtained from day 126 of Napping® with 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The X-axis (dimension 1) accounts for 48,18% of the explained variance, while the Y-axis (dimension 2) accounts for 28,50% of the explained variance. Together the plot accounts for 76,68% of the explained variance in the data set.

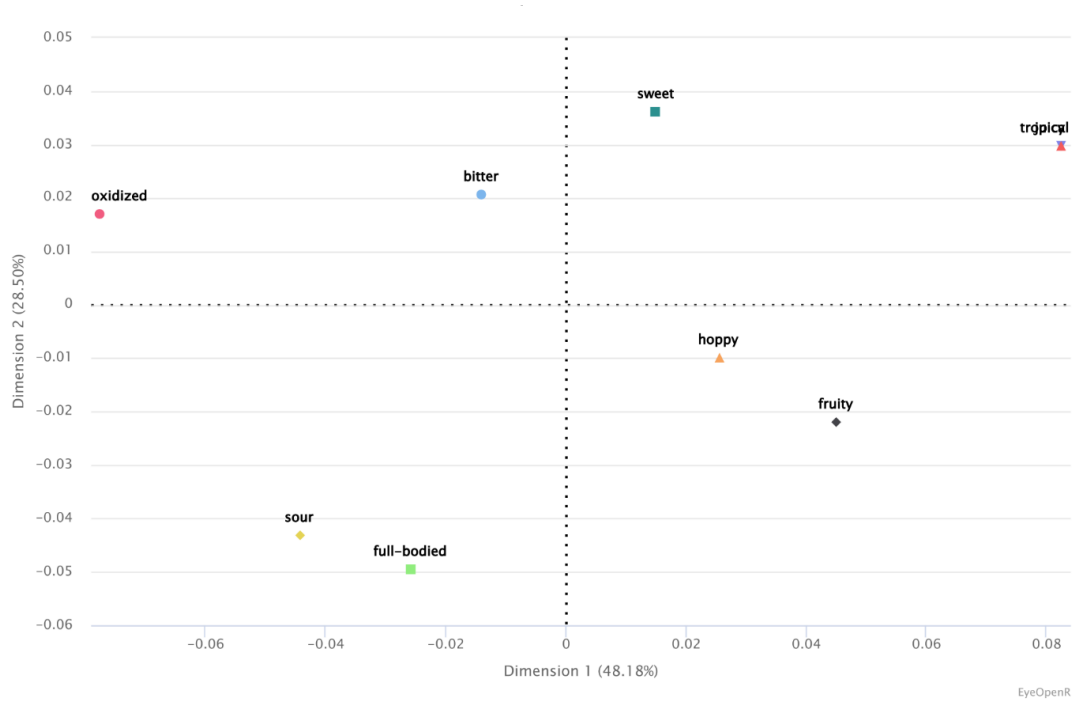

Figure 8 - Two-dimensional CA plot containing descriptors from UFP used by the panelist at a frequency of 5 or more at day 126 of Napping® of 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The descriptor "juicy" is placed beneath "tropical" in the plot. The X-axis (dimension 1) accounts for 48,18% of the explained variance, while the Y-axis (dimension 2) accounts for 28,50% of the explained variance. Together the plot accounts for 76,68% of the explained variance in the data set.

4.3 Changes in color (EBC)

Measuring color spectrophotometrically to look for color changes in the different samples over time, was conducted utilizing the color unit system EBC, which is the European system for the measurement of beer color (Villa, 2011). There was a wish to examine if color changes occurred during the storage time, and if there could be observed any differences with the different additives added.

The results from the color measurements shows that the baseline of EBC-units at day 1 (see Table 4) are pretty similar for all the samples, although P02 is slightly elevated. The biggest changes in color over time occurs from day 1 to day 30. This correlates with the observations of Janish (2019, p. 237), that a darkening of color happens quickly when a NEIPA is introduced to even small amounts of oxygen.

Table 4 – Spectrophotometric analysis of color (EBC) of the seven samples (P01-P07) at day 1, day 30, day 92 and day 126. n=2 for each sample, results reported as mean ± SD.

		Color (EBC)			
Sample	**Variables**	**Day 1**	**Day 30**	**Day 92**	**Day 126**
P01	Beer without additions	8.79 ± 0.04	9.96 ± 0.01	10.30 ± 0.04	10.41 ± 0.05
P02	Intentionally oxidized	8.93 ± 0.19	12.39 ± 1.07	13.53 ± 1.22	13.23 ± 0.65
P03	Potassium metabisulfite (SO_2)	8.72 ± 0.02	9.23 ± 0.02	9.42 ± 0.09	9.33 ± 0.09
P04	Potassium metabisulfite (SO_2) + Ascorbic acid	8.75 ± 0.09	9.13 ± 0.04	9.23 ± 0.03	9.21 ± 0.04
P05	Tannin 1 (EnartisTan SLI)	8.75 ± 0.04	10.07 ± 0.08	10.22 ± 0.03	10.35 ± 0.01
P06	Tannin 2 (Enartis Hideki®)	8.78 ± 0.04	10.00 ± 0.02	10.22 ± 0.00	10.32 ± 0.07
P07	Inactivated yeast (Lallemand Pure-Lees™ Longevity)	8.79 ± 0.06	9.86 ± 0.01	9.73 ± 0.09	9.83 ± 0.08

The values indicate that all the samples with additives added (P03-P07) is fairly stable in color between day 30 to 126, with only small changes occurring. The same goes for the sample without any additions (P01). While the intentionally oxidized sample (P02) continues to increase, as well as exhibiting deviations between the cans tested. P02 is the only sample that really stands out by this analysis, which indicates that oxygen exposure has an effect on color, an interaction Callemien and Collin (2007), Habschied et al. (2020) and Gribkova et al. (2022) ties to the oxidation of phenolic compounds. A longer storage time could help to shed more light on the effect of the additives in terms of color changes.

There is also spotted a trend that both samples with SO_2 added (P03 and P04) having lower color values than the rest at day 30 to 126, as well as increasing very little from day 1 to day 30 compared to the rest. Sulfites are known to have a bleaching effect on anthocyanins in wine (Alcalde-Eon et al., 2019; Carrascón et al., 2018; Han et al., 2021), but hard to find similar occurrences in literature related to beer.

4.4 NMR spectroscopy

There was a desire to examine if any trends or categorical differences could be spotted by NMR between the samples throughout the storage time. Comparing the integrals of the 114 peaks to the TSP integral as reference. The data was first grouped by timeframes to look for trends at a broad view, to compare the seven different samples (P01-P07) at the four different timeframes sampling was conducted during storage.

By looking at the PCA score-plots of the NMR data in Figure 9 (one plot contains 95% confidence regions, while the other contains sample names), it's apparent that changes are occurring in the samples over time. The samples at day 1 are clustered together, indicating a low variance between the samples, apart from one outlier (P01A) which lies outside the 95% confidence region of the group and that we will get back to later. Abbreviations are explained in Figure 9. Painting with a broader brush, the confidence regions of each group are gradually increasing in size from day 1 to day 126, and the samples are more spread out in the plot, indicating an increase in variance between the samples. In other words, the differences between the samples in each group are seemingly increasing over time, compared to the samples from day 1, which shared a higher similarity.

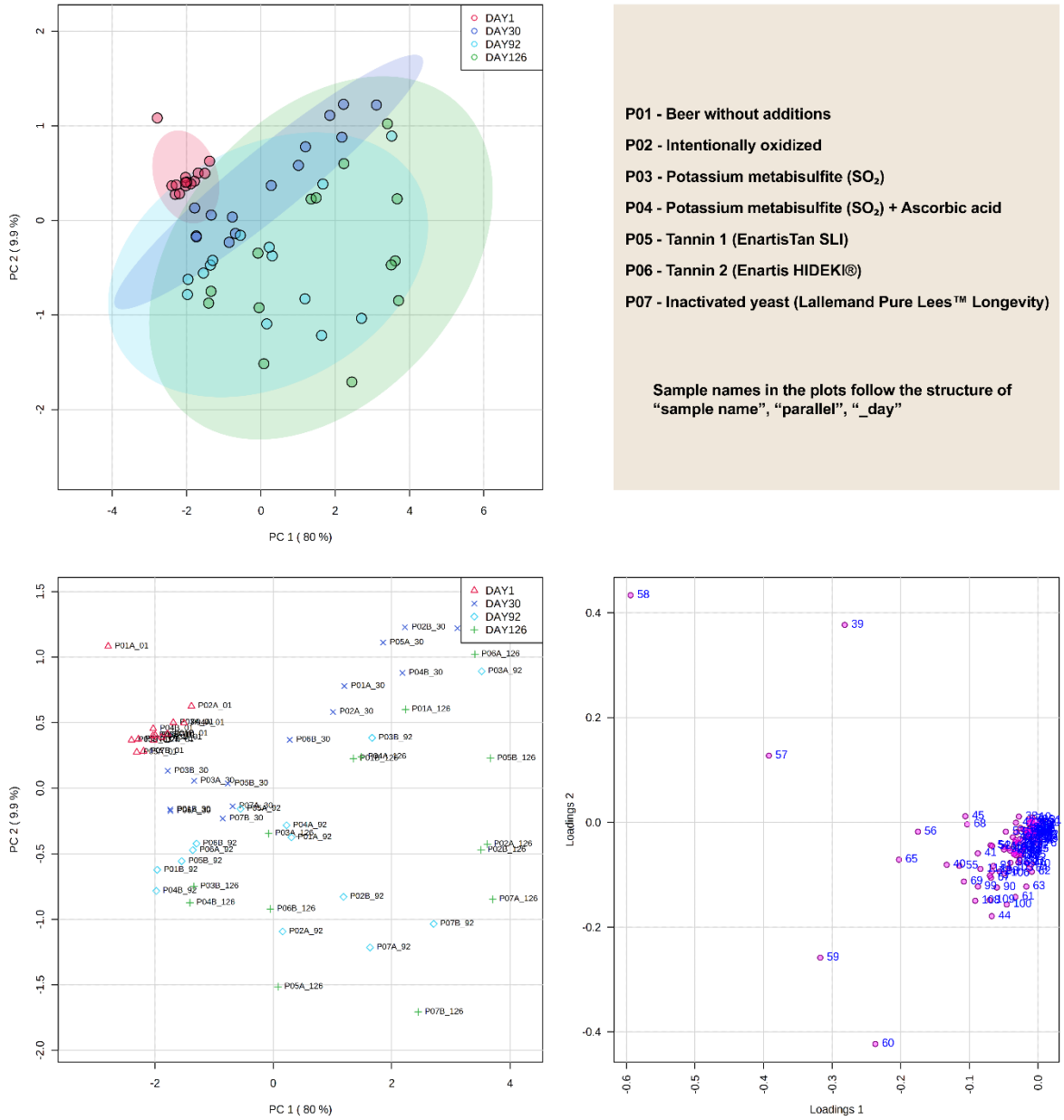

Figure 9 - PCA score-plots (one with 95% confidence regions and one with sample names) and its related loading-plot for all the 56 samples (7 samples, n=2, 4 timeframes) analyzed with NMR, grouped by day 1, day 30, day 92 and day 126 of the shelf stability test. All plots presented in the figure are pre-treated with Pareto scaling. Abbreviations are listed and explained in the figure. The numbers in the loadings plot are the numbers given the 114 extracted data points from the NMR-spectra. The X-axis (principal component 1) accounts for 80,0% of the explained variance, while the Y-axis (principal component 2) accounts for 9,9% of the explained variance. Together the plot accounts for 89,9% of the explained variance in the data set.

Trying to find out what these differences occurs from, we're first looking at the related loading plot in Figure 9. The loadings can tell which peaks that are causing the variance and sample separation in the score-plot. Most peaks are clustered around 0 on both the X- and Y-axis of the loading plot, and do not have a lot of influence on the component. However,

there are some very clear outliers (peak 39, 57-60), as well as other peaks moving away from the big cluster and toward the outliers, which are contributing more to the variance in the data set. The higher the absolute value (towards -1 or 1), the more the peak (variable) influences that component (Taskesen, 2022).

We know there occurred changes to the samples during the storage time, but not what the changes were. Pinpointing if any of the samples differentiate more or less than the rest, were however harder to observe. Looking at the score-plots (see Figure 10) of each day separately, together with their related loading-plots (see Figure 11), the differences between the samples at each point in time of storage should be easier to observe. See Figure 9 for an explanation of the abbreviations. Starting by looking at the score-plot of day 1, the plots consist of seven sample groups (P01-P07) containing two parallels (A and B). At this point in time, a differentiation between the samples in terms of treatment/additives should be possible to observe, as too many chemical changes of the beer itself shouldn't have occurred. There can be observed some clustering of each of the sample groups, P05, P06 and P07, while the rest show some variance between the parallels. Most of this variance is explained in the Y-axis, which only contains 23,5% of the explained variance in the data set, and is necessarily not indicating big differences. However, what takes most of the attention is the outlier (P01A) mentioned earlier. The outlier is one of the parallels of the untreated sample and is most likely caused by a sample preparation error or an error analyzing that sample.

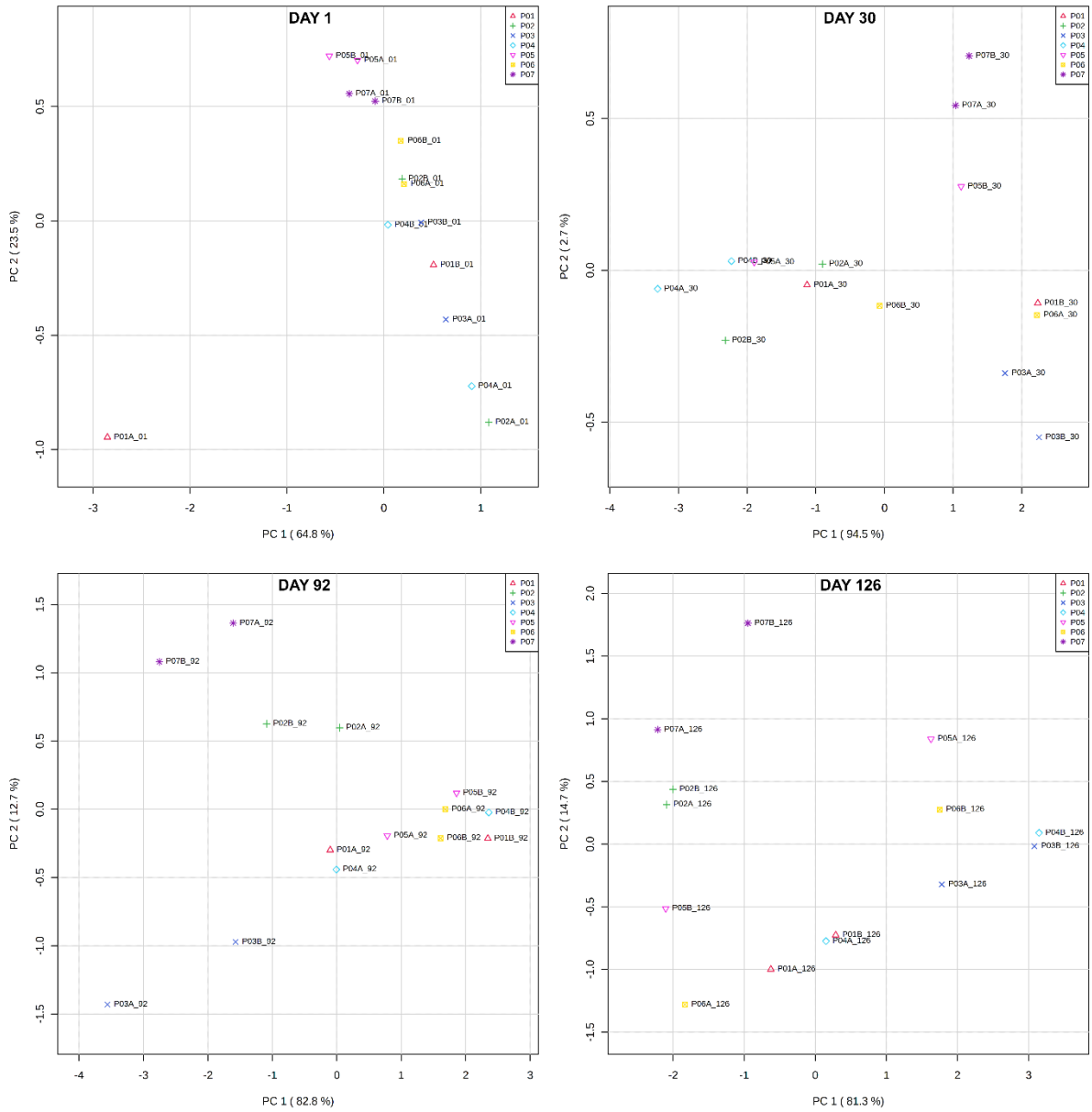

Figure 10 - PCA score-plots from day 1, day 30, day 92 and day 126 of storage time, with samples as groups. Each plot consisting of 14 samples (7 samples, n=2) analyzed with NMR. All plots presented in the figure are pre-treated with Pareto scaling.

At day 30 more spreading in the plot can be observed (see Figure 10), yet some of the sample groups (P03, P07 and somewhat P04) are still showing somewhat concurrence between the parallels (the X-axis explains 94,5% of the variance). At this point in time, variables like net volume of the cans and dissolved oxygen content in each can could have influenced oxidative changes between the samples, causing both variability between parallels and between the sample groups. Nevertheless, it's important to note that we're looking at relative differences here, as we're not looking at identified compounds and know what causes the variability.

Not much is changing by looking at day 92 and day 126 (see Figure 10), and there are still inconsistencies in terms of clustering of parallels, as well as no apparent patterns or trends. Looking at their related loading plots in Figure 11, the same set of peaks are causing the variance and sample separation in the score-plots throughout, although there is more separation in the loadings for day 1 and day 30.

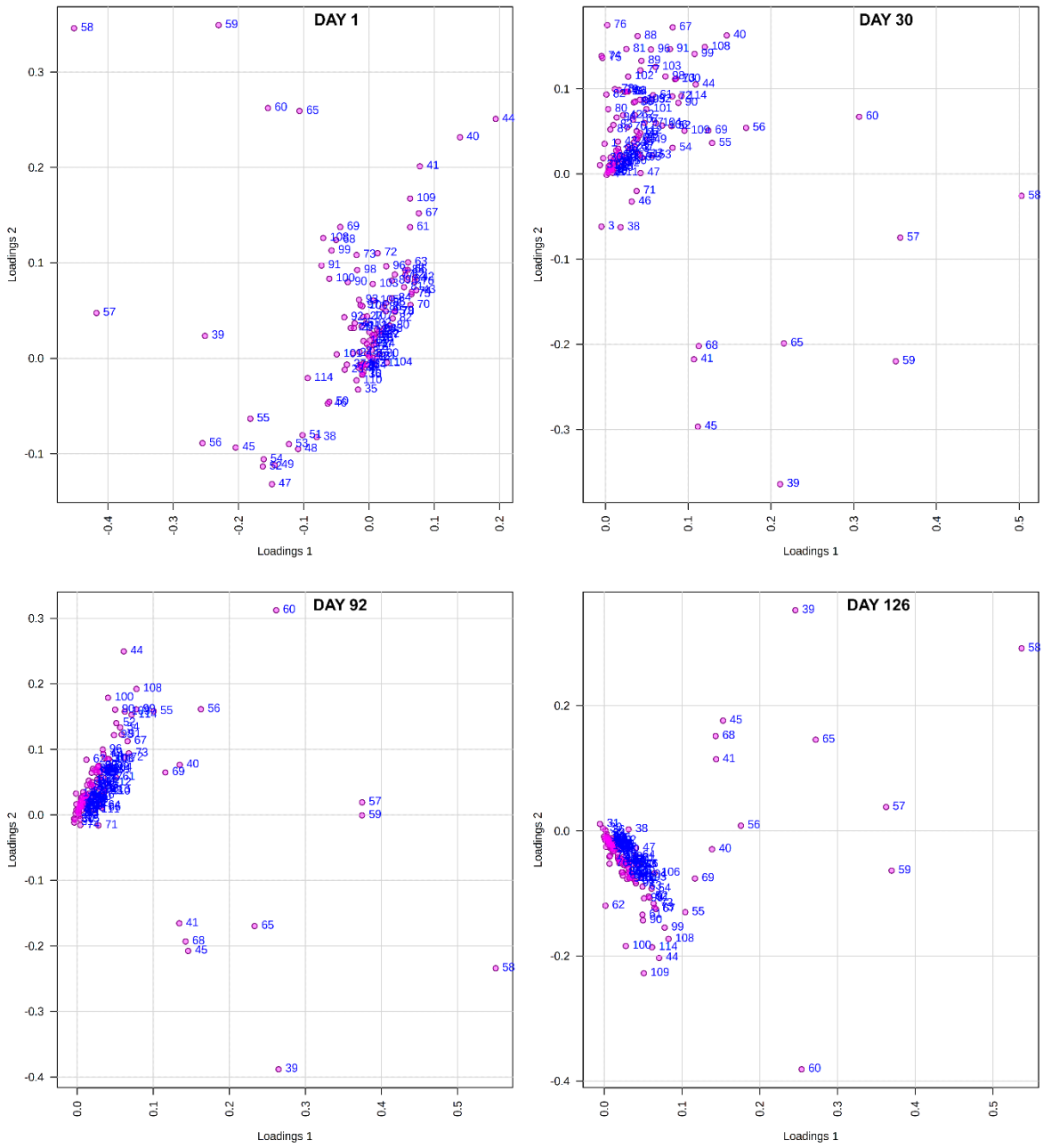

Figure 11 - PCA loading-plots from day 1, day 30, day 92 and day 126 of storage time, with samples as groups. Each plot consisting of 14 samples (7 samples, n=2) analyzed with NMR. All plots presented in the figure are pre-treated with Pareto scaling. The numbers in the loadings plot are the numbers given the 114 extracted data points from the NMR-spectra.

Neither looking at all the samples grouped by timeframes, nor grouped as samples per timeframe, gave much insight in finding out if some samples differentiated more or less than the rest. There was experimented with other methods of data representation (PLSDA, cluster analysis etc.), as well as different scaling methods of the data, to try to improve the results. The experimentation didn't bring anything else to the table, except that the choice of scaling had some effect worth bringing up.

To ensure that high peaks in the spectra doesn't get overestimated when compared to lower peaks, scaling was applied to the data in MetaboAnalyst. The scaling method Pareto scaling (which is mean-centered and divided by the square root of the standard deviation of each variable) were utilized for all the data presented in this results and analysis section. It has the ability to reduce the relative importance of large values, without increasing the influence of the small values so much that it causes noise or false positives (Van Den Berg, Hoefsloot, Westerhuis, Smilde, & Van Der Werf, 2006).

Comparing Pareto scaling with mean scaling, by observing Figure 9 and Figure 12, mean scaling only shows the most prevalent variances (and not necessarily all the important ones). One dense cluster and 6 outliers. While Pareto scaling is causing some more spreading of the cluster, bringing more variations to light, without blowing out the dimensions like methodologies like auto-scaling does (Van Den Berg et al., 2006). Experimenting with both range- and autoscaling, the data set got very dragged out and caused a lot of noise. Van Den Berg et al. (2006) also mentions range scaling being more sensitive to outliers.

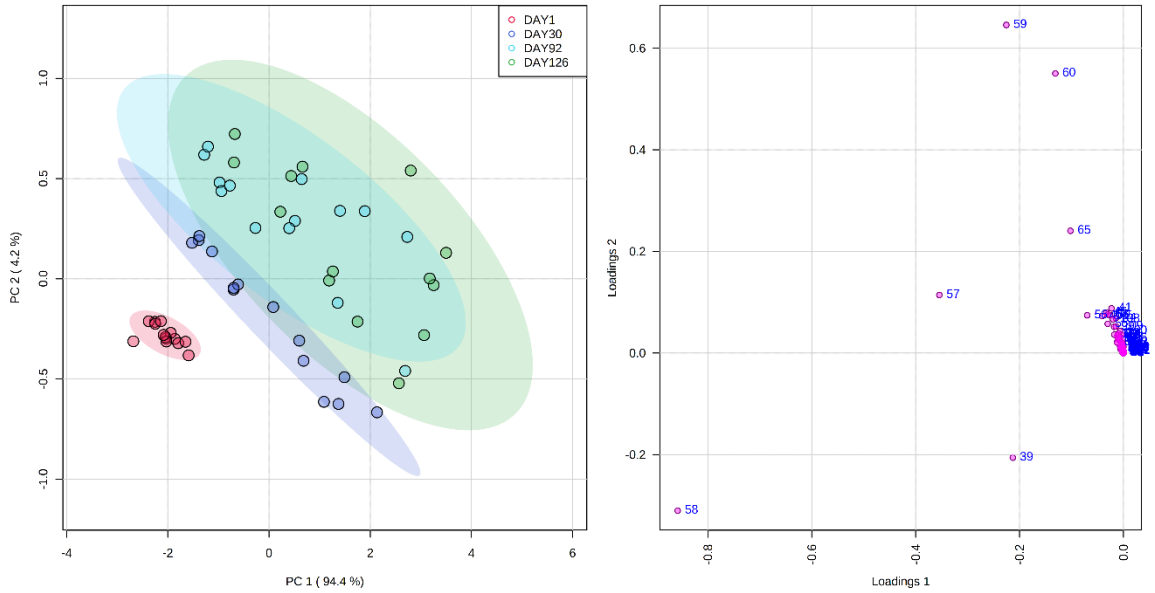

Figure 12 – Mean scaled PCA score-plot (with 95% confidence regions) and its related loading-plot for all the 56 samples (7 samples, n=2, 4 timeframes) analyzed with NMR, grouped by day 1, day 30, day 92 and day 126 of the shelf stability test. All plots presented in the figure are pre-treated with mean scaling. The numbers in the loadings plot are the numbers given the 114 extracted data points from the NMR-spectra. The X-axis (principal component 1) accounts for 94,4% of the explained variance, while the Y-axis (principal component 2) accounts for 4,2% of the explained variance. Together the plot accounts for 98,6% of the explained variance in the data set.

5 Discussion

In this chapter, the addition of different additives and the packaging of the NEIPA, will be briefly discussed. Both in terms of choices that were made and problems that occurred. Afterwards the results presented in chapter 4 will be evaluated in relation to each other and literature, followed by an evaluation of the utilized methodologies in the experiment. Sources of errors that are not presented in the previous chapters, or needs more explanation, will be elaborated upon throughout the chapter. And suggested thoughts and improvements for future studies will be presented at the end.

5.1 Addition of additives and packaging

It was suggested by Guido (2016) that additives either must be able to scavenge peroxides or trap metal ions to be effective as antioxidants in beer. Hence, SO_2 was added to one sample, having both an antioxidative function by being able to react with hydrogen peroxide (Lisanti et al., 2019; Pons-Mercadé et al., 2021), as well as masking oxidative flavors that has already occurred by making carbonyl staling compounds non-volatile (Guido, 2016). As SO_2 itself has a slow reaction directly with oxygen (Pons-Mercadé et al., 2021), a sample with ascorbic acid added as a sacrificial compound (Barril et al., 2016), due to its high oxygen consumptions rates (Pons-Mercadé et al., 2021) was included. Two tannin blends were also selected as additives. An oak-derived tannin blend (EnartisTan SLI) was selected for its capabilities of scavenging oxygen and radicals, in addition to being able to chelate transition metals (Enartis, 2019, p. 57). As demonstrated by Ugliano et al. (2020), oak-derived tannins are able to quickly consume oxygen in wine and had the highest oxygen consumption of the tannins tested. The other blend (Enartis Hideki®) consists of molecular fractions of both gallic, ellagic and condensed tannins, selected for their antioxidative efficiency (Enartis, 2021). A study by De Francesco et al. (2020) demonstrated the protective effect condensed tannins had in beer in terms of flavor stability and color preservation. Lastly, an inactivated yeast product (Lallemand Pure-Lees™ Longevity) was selected for its ability to scavenge dissolved oxygen (Lallemand, 2016), as studies from both Pons-Mercadé et al. (2021) and Lallemand (2021) supported its efficiency in terms of speed of oxygen consumption and total consumption capacity.

The concentration of SO_2 added was decided based on Sacks and Howe (2015) and Waterhouse et al. (2016, p. 142) stating that 20-40 mg/L of free SO_2 is needed to avoid oxidation of wine. No numbers were found for beer. With some expected binding of the addition occurring (AWRI, n.d.-a) and that the media was a 4,47 pH beer (which is higher than typical wine pH), an addition of 60 mg/L of SO_2 was added. The usage rate of 50 mg/L ascorbic acid is taken from a manufacturer that sells it as an antioxidant for beer production (Wray, 2018). The addition of tannins was based on the product declarations and increased a bit further since the media is of higher pH than wine, which the declaration is intended for. The addition of inactivated yeast was also based on the product declaration.

There occurred some unforeseen challenges in canning some of the samples due to the nature of the additives added, causing consequential errors for the rest of the project. Some of the additives were very finely grained and created a lot of small micronucleation sites, which made the beer foam too much in the short amount of time it took the machine to fill and seam the lid on the can. This caused the lid to slip away on the foam, and the seamer crushing the can due to the lid being out of place. Resulting in decreased numbers of cans of sample P05 and P07, as well as lower net volumes (see Table 2) due to the excessive foaming in the cans that made it through the seamer. These two samples were most affected due to their additives' finely grained nature, as well as the usage rate of inactivated yeast (P07) being much higher compared to the other additives.

While canning of the beer was performed on a Friday, the first round of sensory analysis (and the sample preparation for the chemical analysis) was completed on the first working day (Monday) after the weekend. To emulate that the samples was tested at the day of canning, these cans were stored refrigerated (3°C) over the weekend. The changes occurring under these conditions were considered to be negligible, however important to be aware of when evaluating the results.

Choosing to add the additives to the cans is obviously not a viable option commercially, and not the intent either (although none of the additives needs to be filtered out). By doing so, the same beer could be utilized in the experiment, while avoiding variations of splitting and transferring the beer to different containers before packaging. However, there could be benefits or disadvantages by adding the additives earlier in the production process. It could be advantageous if the chelation of metals happened before packaging, leaving precipitated

transition metals in the tank (Enartis, 2021). And it could be disadvantageous if the inactivated yeast were left behind in the tank, as it's supposed to maintain an oxygen scavenging effect for up to 9 months (Lallemand, 2016). In general, it's important to consider the antioxidative function of the additive in question, and how it will affect the product added at a certain point in time.

5.2 Comparison of results from sensory- and chemical analysis

The result from the sensory analysis establishes if there are any sensory differences that can be perceived between the samples of NEIPAs, while the result from the chemical analyses establishes if there are observed any chemical differences between the samples during their storage time. Respectively between an untreated sample, an intentionally oxidized sample and five samples treated with different additives (see Table 1). Together, the results are used to evaluate if there are observed any difference in the state of oxidation between the samples.

There was a desire to detect if any of the additives could affect the product in a sensory aspect. First and foremost, by inhibiting oxidation and maintaining freshness of the NEIPA, but also by being aware that the additives could affect the product's sensory properties directly, either positively or negatively. An additive that helps in terms of oxidation, but adds another off-flavor, wouldn't necessarily be an improvement. There was expected to be found a difference between sample P02 and the rest of the samples, as this sample was oxidized on purpose. The results from both the sensory analysis and the spectrophotometric analysis of beer color (EBC) presented an observed difference, yet the same couldn't be observed in the samples analyzed with NMR spectroscopy. From the sensory analysis P02 were observed to be very different from the rest already from day 30, and the descriptors given from UFP indicated it to be more affected by oxidation than the rest of the samples. The EBC-units from the analysis of color strengthened these findings in relation to P02, as it was the only sample showing a clear trend of an increase in color over time, an occurrence that can be related to beer oxidation (Callemien & Collin, 2007; Gribkova et al., 2022; Habschied et al., 2020; Hodzic et al., 2007).

The sample (P07) with inactivated yeast (Lallemand Pure-Lees™ Longevity) was chosen to be excluded from the sensory analysis, due to the canning irregularities that occurred and was

described in the previous chapter. But there was unexpected that P07 seemingly kept so well in terms of color (see Table 4), and that their EBC-units were stable after day 30, knowing the low net volume and high deviations between the cans, which is presented in Table 2. At this point it would only be speculation, but it could both be an indication of selected inactivated yeast, like Pure-Lees™ Longevity, being as effective in consuming oxygen as Pons-Mercadé et al. (2021) and Lallemand (2016) reports. Or it could be that the intense foaming in the can pushed out most of the oxygen in the headspace. Or a combination. Nonetheless, it would definitely be a contender for further research.

Sensory analysis indicated that all the additives had an antioxidative effect, as both sample placement and descriptors indicated that the sample without any additions (P01) were gradually moving towards the intentionally oxidized sample (P02) in terms of similarity over time. While the ones assessed with additives added, remained constant in relation to P02. It would have been interesting to see when the samples with additives also started moving towards the oxidized sample, so ideally the storage test should have lasted longer. This would have also added some more depth in terms of proving the indication.

The samples with tannins added, P05 (EnartisTan SLI) and P06 (Enartis Hideki®), were the ones that seemingly kept the best in relation to P02 and were also associated with descriptors of how the style is supposed to taste fresh. EnartisTan SLI, which is a tannin blend that is oak-derived, seemed to be slightly more effective of the two. This is consistent with what Ugliano et al. (2020) found when researching different tannins and their oxygen consumption abilities in wine. The producer of EnartisTan SLI, market it as having extraordinary capabilities of scavenging oxygen and radicals (Enartis, 2019, p. 57), as well as being able to chelate metals (Enartis, 2020b). An additive that potentially can chelate transition metals in beer, can help heighten the beer's shelf stability by reducing the formation of ROS (Chrisfield et al., 2020; De Francesco et al., 2020; Gresser, 2009; Guido, 2016). Nevertheless, further research is needed to examine if this is actually occurring. It would be especially interesting to look at manganese, since the production and composition of NEIPAs makes this specific transition metal highly relevant (Janish, 2019, pp. 242-245).

Taking the net volume of the cans (see Table 2) into consideration, it was surprising that P05 was perceived this well from a sensory perspective, as the cans were filled low (423 ± 4,4 ml), due to the nature of the additive added. In consequence it would be expected to have

higher HSO than properly filled cans. No observations from the color measurements or NMR spectroscopy could support these findings.

The findings for P03 and P04 are a bit more inconclusive, as they were perceived similar from day 30 to 126, but only described to be sulfury at day 30. Their consistent placement relative to P02, could both indicate that their oxidative development were considered stable, but also that they were perceived different than the other samples due to another off flavor. More descriptive data would've made it easier to observe what is occurring (this will be more discussed in chapter 5.3.1). In terms of color measurements there was spotted a trend that both samples seemingly had lower color values than the rest at day 30 to 126, although not enough to draw any conclusions by itself. No observations from NMR spectroscopy could support any of these findings.

Only scraping the surface, the NMR analysis conducted doesn't show the same differences as sensory analysis does. But it's important to remember that this thesis doesn't look at compound identification and concentrations of compounds, but at 114 selected peaks in terms of relative differences to the reference TSP, trying to observe patterns and trends. The findings from NMR shows clearer changes occurring to the samples over time, than between the samples at the different timeframes, which makes it look like the storage or aging itself causes the most variation to the data set. There was an aspiration to observe trends or sample clustering like in the sensory analysis, where the intentionally oxidized sample was clearly differentiated from the rest of the samples. In addition to observing trends for the other samples to strengthen or weaken the findings of the sensory analysis. But there was no conclusive evidence, other than variations and loadings got bigger throughout the storage time of the NEIPA. Indicating that the changes occurring, or at least the changes that is observed in the data set, are occurring more related to time than the additives added. And that further investigation into peak identification is needed to get clearer results.

This strengthens the importance of utilizing human sensory impulses and the advantages of using sensory analysis as a tool in combination with chemical analyses when conducting research and quality control (Rødbotten, 2015). However, if the results coincided, that could potentially decrease the need for expensive and time-consuming sensory panels, as well as removing potential issues with sensory bias. To give an example, this study could have

quickly been even more affected by the panel struggling with COVID-19 related loss of smell and taste. But more on that in the next chapter.

5.3 Evaluation of utilized methodologies

5.3.1 Evaluation of the sensory analysis

Napping® with UFP is not an ISO-standardized method, and modifications can be made to make it more suiting for the objective of the analysis (Varela & Ares, 2012). That being the case, appearance was not a subject for this sensory evaluation, as it was quantitatively measured with UV/Vis spectroscopy. Only retro- and orthonasal odor and taste were assessed by the panel. In a perfect world, the sensory analysis would have been conducted in a room with colored glasses and lightning to prevent the assessors from being affected by the sample color and mitigate possible visual bias (Pickup et al., 2018). But this was not possible due to the facilities and equipment available.

Although Napping® with UFP being a rapid sensory method that is used with good success with untrained and semi-trained panels (István & Kókai, 2021), the panels utilized are usually of sizes above 9 assessors (Varela & Ares, 2012). It was hard to include more assessors in this project, as the brewery panel at E.C. Dahls only consists of 6 people. It was difficult to find a larger brewery-related panel within the limitations of the thesis, and generally difficult gathering panels during the pandemic because of its accompanying restrictions. It didn't make it easier that this was a shelf stability test, which caused the need of each panel member being available for every sitting conducted over a period of 4 months. At multiple occasions panel members had to stay home in quarantine or got sick, and sessions had to get postponed. After three cancellations, the third session was conducted at day 92 with one panel member missing, as he called in sick while the sample preparation was taking place. By the point of day 92, two of the panelists had reported impaired sense of taste and smell due to recent COVID-19 infections. The third panelist affected (the panel member who called in sick on day 92) reported that he didn't feel impaired from a sensory perspective for the last session conducted at day 126. As the panel leader, and based on the results, there wasn't observed any decline in the quality of data received for day 92 and 126. Rather the other way around, most likely as the panel got more familiar with Napping® with UFP, and the samples in question.

The time limit of the thesis, but more importantly the time restrictions of the panel, and the unpredictableness of the COVID-19 pandemic, kept a restraint on how well trained and calibrated the panel could be before the main sensory assessment. Ideally it should have been spent even more time on getting the panel familiarized with the methodology and the beer style (István & Kókai, 2021), and they should have been more calibrated on placement and utilization of a more similar terminology in terms of descriptors, to get even more consistent results (Moss & McSweeney, 2022; Perrin et al., 2008). This can be reflected in the problems and uncertainties that occurred in the first session of utilizing Ultra Flash Profiling (UFP), resulting in a panel meeting prior to the next session, to calibrate and unify the panel. Although no descriptors were included in the results for day 1, there were not expected to be observed differences in terms of oxidation this short after canning. But it would have been interesting to have more descriptive data of the NEIPA with the additives added, before oxidation started occurring, to get a clearer picture of the sensory influence of the additives themselves.

If indeed sample P03 and P04 shared an off-characteristic sulfur note, as brought up in chapter 4.2 and 5.2, it is possible that this difference would have gotten more attention by the panelists than the sample's oxidative state, hence it could be placed on that premise too. But having used a rapid sensory method that looks at the broader level of details (István & Kókai, 2021), and not being as good as picking up nuances and details as the traditional descriptive methodology (Perrin et al., 2008) with a fully trained panel (Varela & Ares, 2012), it's hard to pinpoint the cause of difference for these two samples. Still, it's worth noting that Napping® with UFP might be more in touch with the mainstream consumer, as sensory analysis with traditional descriptive methodology does not necessarily look at the qualities the consumer consider to be important (István & Kókai, 2021). In the end, it's the consumers that ends up deciding if a product is acceptable or not.

With the limitation of the panel, thesis and especially the COVID-19 situation, Napping® with UFP was the most suited methodology. It was considered to be satisfactory knowing if, and when, changes occurred, without necessarily knowing exactly what happens. A traditional descriptive method would have been better at describing differences occurring with words (Moss & McSweeney, 2022; Perrin et al., 2008), but the time-consuming effort of both extensively training and conducting sensory analysis over time, would not have been

feasible under these circumstances mentioned. Both Moss and McSweeney (2022), and Varela and Ares (2012) confirms how time-consuming descriptive methodology is, and that it can be problematic for research with short timeframes or limited usage of the panelists.

5.3.2 Evaluation of the chemical analyses

UV/Vis spectroscopy was never intended to give a lot of results on its own, but to objectively quantify color change and have a supplemental function to sensory analysis and NMR analysis.

Even by knowing the results from the sensory analysis, and thus potentially knowing what to look for with NMR spectroscopy, it was hard to observe any categorical differences or trends throughout the storage time of the samples. Not getting clear separation between the sample groups, due to the variation between the parallels of samples, could be caused by sample preparation errors, like pipetting errors. But variables like net volume of cans and dissolved oxygen content could also have caused variation between the samples, due to inconsistencies in oxidative changes between the parallels. Significance could also be hidden by background noise, however this was attempted to be decreased by utilizing Pareto scaling, as well as other methods of data representation, as talked through in chapter 4.4.

Although, no conclusive evidence on sample level was gathered from the NMR analysis, peaks of interests could be inspected down to sample level and compared to other samples. But without knowing if the peaks are related to oxidation, and no real trends that could narrow the search were observed, it was beyond the focus of this thesis.

5.4 Future perspectives

The results from the thesis are preliminary and can hopefully lead the way for more detailed and comprehensive studies where either the sensory aspect or the aspect of chemical analysis is the focus. For example, in terms of compound identification and quantification by NMR, or more thorough descriptive sensory analysis with a fully trained panel.

It could be interesting to look at a similar shelf stability experiment that ranges over a longer period. Even with the same additives, it would be interesting to see if the results could be recreated and the descriptive detail increased. As the preliminary results from Napping® with UFP and UV/Vis spectroscopy indicated that the storage time wasn't long enough to consider the total effect of the additives.

Considering the sensory results, enological tannins are the most promising contender for further research. However, inactivated yeast, like Lallemand Pure-Lees™ Longevity, is also very much worth prioritizing. In terms of how promising the background information presented in chapter 2.3.4 looks (very high oxygen scavenging capacity over a long period of time). But also due to the limited results gathered from this thesis. Being able to keep its color that well after being severely low filled is worth looking into.

Utilizing biological additives like inactivated yeast, and tannins, are probably more accepted by the consumers these days, as inorganic compounds like SO_2 has gotten a bad reputation by its extensive use in the wine industry. These biological additives do not require any labelling information and are not linked to allergic- and sensitivity reactions, as far as I have gathered. It's at least worth taking this into account if additives are considered used commercially in NEIPAs.

Lastly, it would be productive to look more into options for reducing transition metals in NEIPAs and examining the effect. For instance, by examining chelating effects of additives by tracking transition ions with inductively coupled plasma mass spectrometry (ICP-MS) or another fitting method of analysis. By using tannins like in this experiment, or with the addition of other additives not covered by this thesis. For example, the bioactive polymer chitosan which has been linked to both chelating- and antioxidative function in wine research (Castro Marín, Colangelo, Lambri, Riponi, & Chinnici, 2021; Castro Marín et al., 2019; Robillard, 2021).

6 Conclusions

This thesis aimed to improve the shelf stability of New England IPAs, by observing whether any particular additives introduced at packaging were able to slow down the oxidation of the final product. It's difficult to draw conclusions from the experiment conducted, and the results should be considered as preliminary. However, the results from the sensory analysis indicated that all samples with additives added, kept better than the one without, hence slowing down oxidation. The addition of enological tannins, especially those derived from oak (EnartisTan SLI), had the most effect of all the additives on the NEIPAs' oxidative stability. Still, the storage time needs to be longer to consider the total effect of the additives, and it has to be taken into account that the sample with inactivated yeast was omitted from the sensory analysis due to a source of error.

The results from the supporting chemical analyses were inconclusive, and the main takeaway from the analysis of color was that it proved that darkening of color occurred in the intentionally oxidized sample, and that it occurred quickly. It did not, however, correlate with any of the other observations from the sensory analysis. NMR spectroscopy provided limited insight and could not discriminate on utilized level if single samples differed more or less than others. The only pattern observed was that the difference between samples in general increased the longer they were stored, and that the samples shared a higher similarity at day 1, before storage.

This thesis supports that additives could be another tool in the toolbox for brewers striving to increase shelf life of their NEIPAs. Nevertheless, more work on the topic is needed, in terms of more detailed and comprehensive studies that extend over a longer period of time. Not only in regard to efficiency, but also in terms of when the different additives are added in the production process.

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A list of equipment, ingredients, additives and chemicals used for both the production and packaging of the beer itself, and for the analyses performed throughout the shelf stability test.

Beer production and packaging:

Equipment:

- Malt mill
- Hot liquor tank
- Mash tun (w/ rake and lautering sieve)
- Kettle
- Plate chiller
- Pump
- Fermentation vessel (FV) – 2000L with cooling jackets and coned bottom.
- Brite tank – 2000L with cooling and carbonation stone
- Tank and regulator for CO_2
- Carbon dioxide (CO_2)
- Thermometer
- Canning machine (Twin Monkeys Gunnison)
- Cans and lids (Crown – 440 ml)
- Mettler Toledo Analytical Balance AB54-S (0,1 mg – 4 decimals)
- Calibration weight (50 g)
- Weighing boats (sterile, plastic)

Ingredients:

- Water
- Malt
    - Thomas Fawcett - Golden Promise
    - Stangeland Mølle - Flaked oats
    - Thomas Fawcett - Torrified Wheat
    - Weyermann - Carapils
    - Weyermann - Wheat malt
    - Weyermann - Melanoidin
- Yeast
    - The Yeast Bay - Hazy Daze
- Hops
    - CO2-extract (CTZ - 60,0% AA)
    - Tettnanger (5% AA)
    - Centennial (9,1% AA)
    - Chinook (11,2% AA)
    - Mosaic (11,6% AA)
    - Citra (14,5% AA)
    - Citra LupuLN2 (CRYO) (23,9% AA)
    - Mosaic LupuLN2 (CRYO) (21,3% AA)
- Additives in production
    - Zincsulfate
    - Calcium chloride
    - Calcium Sulfate
    - Natrium Chloride
    - MgSO4
    - Lactic Acid
    - WHC Lab Anti Foam
- Cleaning and disinfection chemicals production:
    - Caustic (NaOH)
    - Peracetic acid (PAA)

Additives added at canning:

- Potassium metabisulfite (E224) (Enartis Winy)
- Ascorbic Acid (Sigma-Aldrich L-Ascorbic acid 99%)
- EnartisTan SLI
- Enartis Hideki®
- Lallemand Pure-Lees™ Longevity

Sensory analysis:

- Duni 30 cl Tumblers (Transparent plastic glasses)
- Erlenmeyer flask (2L)
- Biscuits (Sætre Kaptein)
- Water
- Parafilm

Chemical analyses:

- Pentair Haffmans c-TPO (TPO at E.C. Dahls)
- Radiometer Copenhagen PHM92 LAB and Hach Radiometer analytical Red Rod Combined pH Electrodes (pH-meter at E.C. Dahls)
    - 4.01 and 7.00 pH buffer solution

Equipment (UV/Vis spectroscopy):

- Biochrom Libra S22 UV/Vis spectrophotometer
- Cuvette (HelmaAnalytics OS High Precision Cell (10 mm) - art no. 100-10-20)
- VWR 3.3 250 ml Erlenmeyer flask
- Heidolph unimax 2010 platform shaker
- BD Plastipak Syringe with Luer Lock 10 ml (sterile)
- VWR 25 mm Syringe Filter (w/ 0,45 µm Polyethersulfone membrane)

- VWR Centrifuge Tube (15 ml, conical bottom) - art. nr: 525-1070
- Thermo Scientific Finnpipette Fixed 10 ml (FJ02976 4501)
- Thermo Scientific Finntip 10 ml
- Distilled water

Equipment and reagents (NMR):

- Syringe
- Sterile filter (0,2 µm, 25 mm diameter, luer lock (female)
- Eppendorf tube (5,0 ml)
- Eppendorf tube (1,5 ml)
- 5 mm NMR-tubes with tube caps (Bruker LabScape™ Stream 5)
- Sample holder for NMR-tubes (Bruker LabScape™)
- VWR Ultrasonic cleaner
- Vortex mixer
- Eppendorf Research® Plus Adjustable Volume pipette 100-1000 µl
- Eppendorf Research® Plus Adjustable Volume pipette 20-200 µl
- Pipette tips - 1000 µl and 200 µl
- Buffered stock solution (500 mM sodium phosphate pH 7.4 buffer containing D_20 (99%) and TSP (1%).
- Bruker AVANCE III HD 800 MHz NMR spectrometer
- Bruker SampleJet

Method: **All Grain** Style: **American IPA** Boil Time: **60 min** Batch Size: **2450 liters** (ending kettle volume)
Pre Boil Size: **2000 liters** Pre Boil Gravity: **18.2 °P** (recipe based estimate) Efficiency: **79%** (ending kettle)
Calories: **187 calories** (Per 330ml) Carbs: **16.9 g** (Per 330ml)

Original Gravity:	Final Gravity:	ABV (standard):	IBU (tinseth):	EBC (ebcmorey):	Mash pH	Cost $
15.0 °P	**3.0 °P**	**6.5%**	**34.7**	**10.9**	**5.7**	**n/a**

Fermentables

Amount	Fermentable	Cost	PPG	EBC	Bill %
375 kg	United Kingdom - Golden Promise		37	6.51	59.3%
120 kg	Flaked Oats		33	4.37	19%
50 kg	Torrified Wheat		36	3.84	7.9%
50 kg	Weyermann CaraPils		35	4.37	7.9%
25 kg	Weyermann - German - Wheat Malt		37	2	4%
12.50 kg	Weyermann Melanoidin		35	70.55	2%

632.50 kg / $ 0.00

Hops

Amount	Variety	Cost	Type	AA	Use	Time	IBU	Bill %
100 g	co2-extract		Extract	61.1	Boil at 100 °C	60 min	4.6	0.3%
2,000 g	Tettnanger		Pellet	4.5	Boil at 100 °C	5 min	1.48	5.6%
3,000 g	Centennial		Pellet	10	Whirlpool at 85 °C	0 min	9.8	8.4%
2,500 g	Chinook		Pellet	13	Whirlpool at 85 °C	0 min	10.61	7%
2,000 g	Mosaic T90		Pellet	12.5	Whirlpool at 85 °C	0 min	8.16	5.6%
10,000 g	Centennial		Pellet	10	Dry Hop	6 days		28.1%
5,000 g	Citra T90		Pellet	11	Dry Hop	6 days		14%
5,000 g	Yakima Chief Hops - Citra LupuLN2 (Cryo)		Lupulin Pellet	25	Dry Hop	6 days		14%
5,000 g	Mosaic T90		Pellet	12.5	Dry Hop	6 days		14%
1,000 g	Yakima Chief Hops - Mosaic LupuLN2 (Cryo)		Lupulin Pellet	22	Dry Hop	6 days		2.8%

35,600 g / $ 0.00

Mash Guidelines

Amount	Description	Type	Temp	Time
		Infusion	67 °C	--

Starting Mash Thickness: 3 L/kg

Other Ingredients

Amount	Name	Cost	Type	Use	Time
4 g	zinksulfat		Other	Boil	10 min.

Yeast

- The Yeast Bay - Hazy Daze

Amount: 1 Each **Cost:** **Attenuation (custom):** 80% **Flocculation:** Low
Optimum Temp: -18 - -18 °C **Starter:** No
Fermentation Temp: 21 °C **Pitch Rate:** 0.35 *(M cells / ml / ° P)* 12883 B cells required

Notes

Gypsum/CaSO4: 430 g
Epsomsalt/MgSO4: 300 g
Table salt/NaCl: 300 g
Calcium Chloride/CaCl: 430 g
Lactic acid: 1300 g

	#197	Beer D	Tank 3	Date/brewer	30/9
OG: 15.3 plato	**PH pre-ferment**				
FG: 3.5 plato	**PH packaging**				
ABV: 6,39%	**Carb. volume**	2,6 vol/co2			
			Start time	End time	
HLT temp OK? Turn off element	Ok				
Water mash-in, volume	1825L	Mash-in	9:20	10:40	
Water adjustment	Ok				
Mash temp	66,3C				
Mash PH	5,15 pH				
Sparge volume					
Preboil volume	2200L	Wort transfer	10:45		
SG 30 min boil	18.5 plato	Boil			
Hop extract/zinc sulphate/whirlfloc	Ok				
Hops, 5 min	-				
Dilution L	450L				
Lactic acid addition	Ok				
Hops whirlpool	Ok				
WP temp	88C				
Postboil L	2650L	2450 +/- to Fermentation Vessel (FV)			
KO temp	20C	Transfer to FV	14:50		
O2 Time	50%				
Yeast pitch kg, gen., harvested from	From #195 - Gen.3		Notes		
Fermentation temp	20C		150 g NoFoam added to FV		
Dryhop 1 date	10/10-2021				
Dryhop 2 date	-		4/10 - 4 plato		
Rousing hops	-				
Hops dropped	15/10-2021				
Soft-crash date	-				
Harvest yeast	-				
Cold-crash date	15/10-2021				
Packaging date	22/10-2021				
No. of kegs	50				
No. of cans (trays)	79				
No. of bottles					
Closing checklist after brewday					
HLT on?					
Fermenter closed, pressure valve open?					
Yeast pitched?					

Day 126

Step 1: **Place the 6 samples** on the screen according to their **differences** and **similarities**. If two samples are perceived different, they are placed far away from each other. If two samples are perceived similar, they are placed together or close to each other.

How far or close they are to each other will determine the grade of difference.

Step 2: After all the samples are placed, you may give descriptors you feel differentiate single samples or groups of samples. To do this, **draw an area** around one or more samples you feel share the same descriptor(s), and **write the descriptor(s)** in the text box that appears, each one separated by a comma.

Taste order

You've got 6 samples in front of you to assess in terms of flavor (**taste + aroma**). The samples are identified by 3-digit codes. Assess them first in the order that appears on the screen. Afterwards you can revisit the samples in whichever order you please.

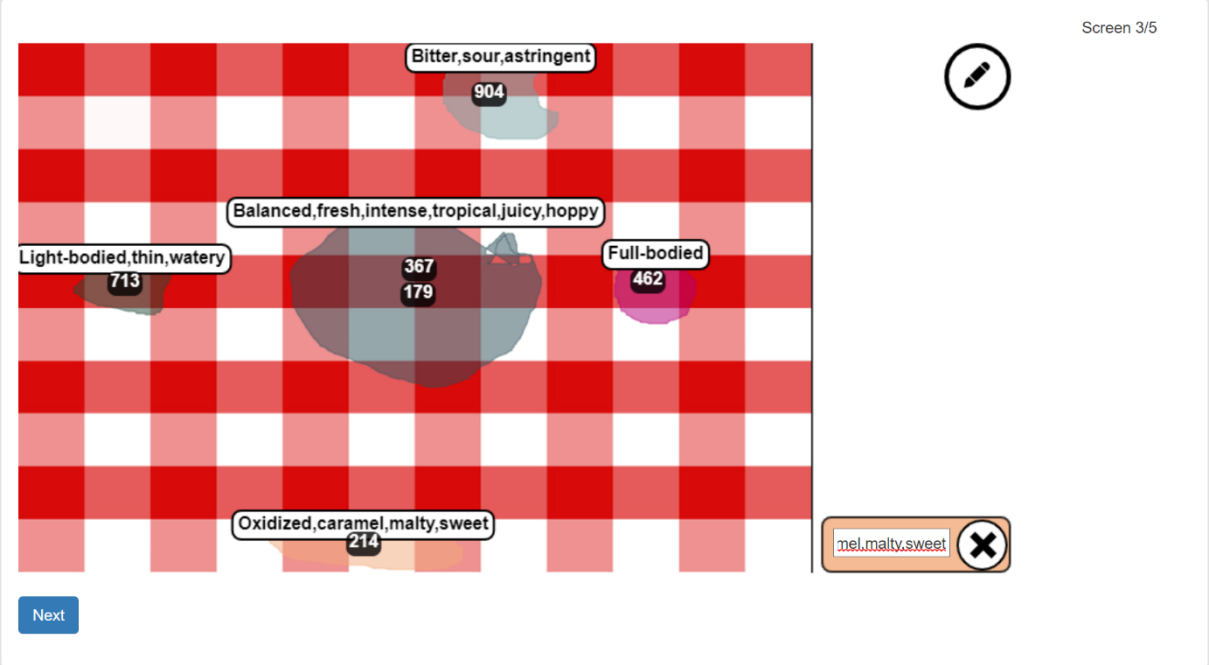

Figure 13 - Example of how Napping® with UFP is conducted in EyeQuestion®. This is not a real assessment, only an example.

LIST OF DESCRIPTORS

You are NOT limited to these words, but if you wish to describe something that coincides with this list, please use the descriptors below.
But you can also add others!

Checklist:

- Remember to remove "region" when entering the descriptors.
- Use comma between each descriptor (not dots or semicolons).

Descriptors:	**Explaination:**
Bitter	Basic taste - Bitterness
Sweet	Basic taste - Sweet as sugar/honey
Sour	Basic taste - Acidic, sharp
Malty	Sweet, malt-derived flavors
Full-bodied	Thick and viscous mouthfeel
Light-bodied	Watery and low viscosity mouthfeel
Balanced	Overall balance between the beers components
Fresh	Fresh and bright hop character
Caramel	Sweet as caramel
Alcoholic	Burning, hot sensation, higher/fusel alcohols
Oxidized	Caramelly, papery, not fresh, old hops, sherry
Tropical	Taste/aroma of tropical fruit
Fruity	Taste/aroma of fresh fruit
Mild	Low taste/aroma intensity
Intense	High taste/aroma intensity
Vegetal	Green, astringent notes - Like hop matter
Vitamin-like	Taste/aroma reminiscent of vitamins
Sulfury	The smell of a recently lit match
Juicy	Smooth like fruit juice
Hoppy	High hop intensity / Hop-forward

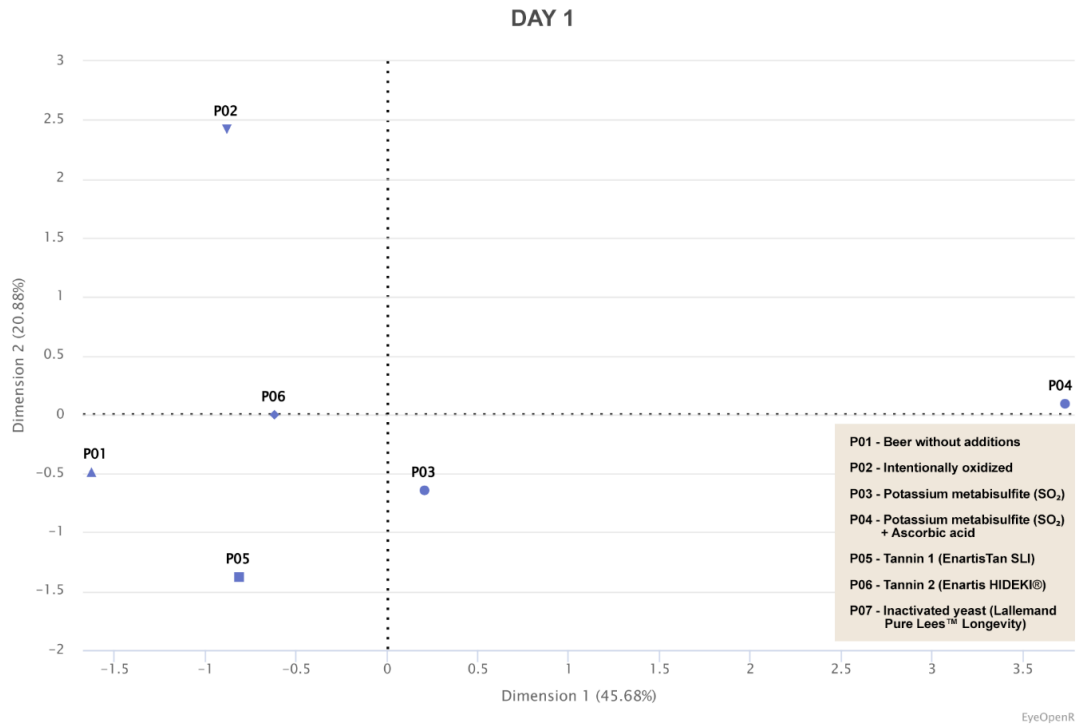

Figure 14 - MFA plot obtained from day 1 of Napping® with 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The X-axis (dimension 1) accounts for 45,68% of the explained variance, while the Y-axis (dimension 2) accounts for 20,88% of the explained variance. Together the plot accounts for 66,56% of the explained variance in the data set.

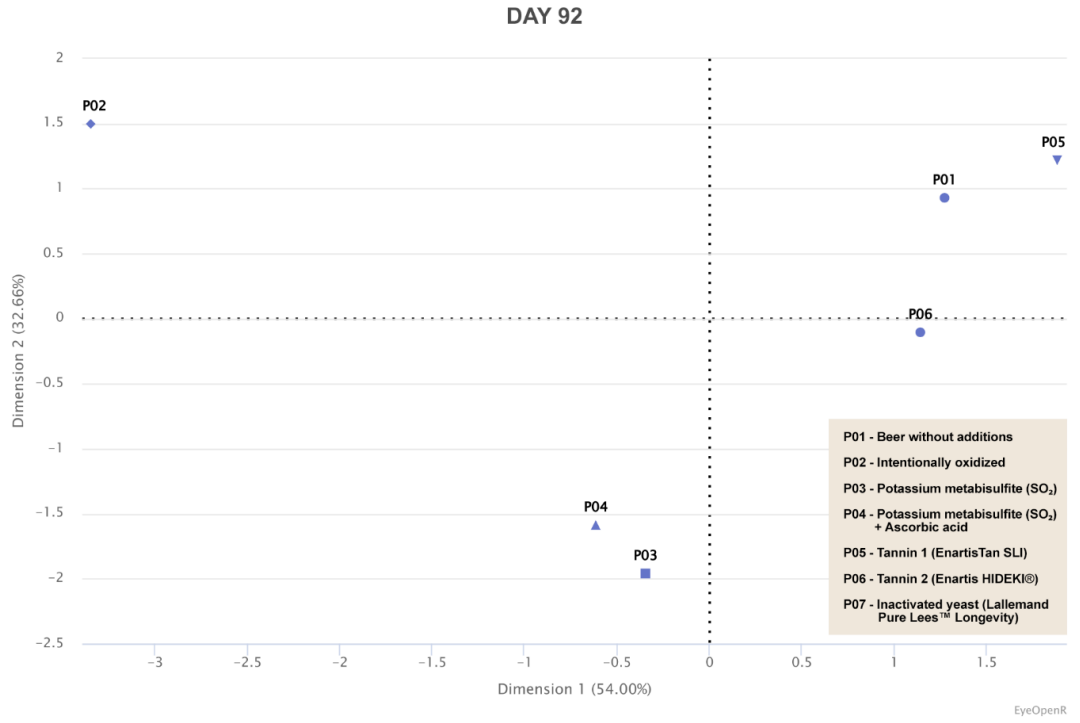

Figure 15 - MFA plot obtained from day 92 of Napping® with 6 different samples (P01-P06). The panel consisted of five semi-trained assessors. The X-axis (dimension 1) accounts for 54,00% of the explained variance, while the Y-axis (dimension 2) accounts for 32,66% of the explained variance. Together the plot accounts for 86,66% of the explained variance in the data set.

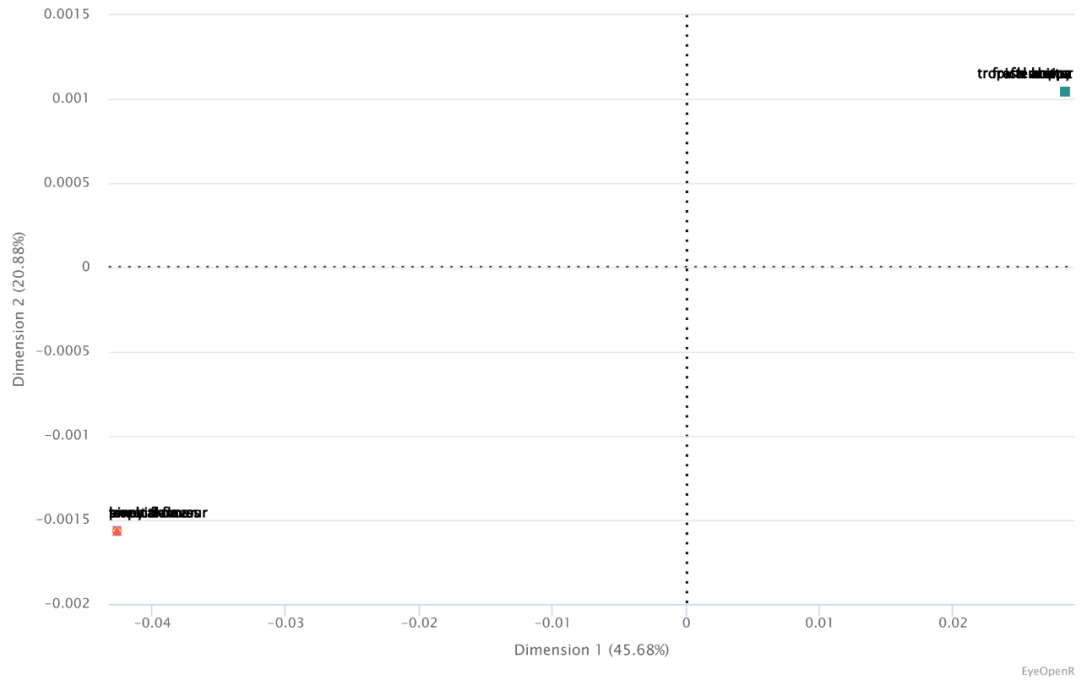

Figure 16 - Two-dimensional CA plot containing descriptors from UFP used by the panelist at a frequency of 5 or more at day 1 of Napping® of 6 different samples (P01-P06). The panel consisted of six semi-trained assessors. The X-axis (dimension 1) accounts for 45,68% of the explained variance, while the Y-axis (dimension 2) accounts for 20,88% of the explained variance. Together the plot accounts for 66,56% of the explained variance in the data set. The red dot of overlapping descriptors contains: "tropical flavor, low bitterness and sweet". The blue dot contains: "tropical aroma, hoppy, fresh".

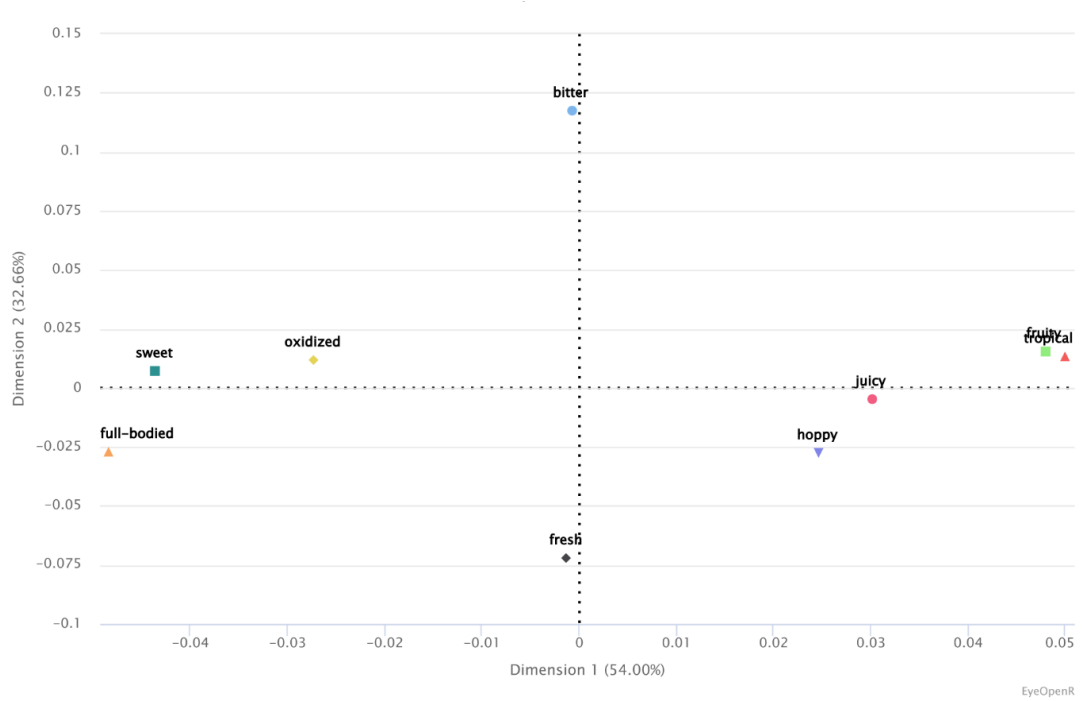

Figure 17 - Two-dimensional CA plot containing descriptors from UFP used by the panelist at a frequency of 5 or more at day 92 of Napping® of 6 different samples (P01-P06). The panel consisted of five semi-trained assessors. The X-axis (dimension 1) accounts for 54,00% of the explained variance, while the Y-axis (dimension 2) accounts for 32,66% of the explained variance. Together the plot accounts for 86,66% of the explained variance in the data set

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DISTRIBUTION OF FREE SO_2

FROM pH 3.0 – 4.0 (PKA = 1.81)

pH	% Molecular SO_2 (m)	% Bisulfite (HSO_3-)	% Sulfite (SO_3=)	Minimum ppm of Free SO_2		
				0.8 molecular	0.5 molecular	0.3 molecular
3.00	6.1	93.9	0.012	13	8	5
3.05	5.3			15	9	6
3.10	4.9	95.1	0.015	16	10	6
3.15	4.3			19	12	7
3.20	3.9	96.1	0.019	21	13	8
3.25	3.4			23	15	9
3.30	3.1	96.8	0.024	26	16	10
3.35	2.7			29	18	11
3.40	2.5	97.5	0.030	32	20	12
3.45	2.2			37	23	14
3.50	2.0	98.0	0.038	40	25	15
3.55	1.8			46	29	17
3.60	1.6	98.4	0.048	50	31	19
3.65	1.4			57	36	21
3.70	1.3	98.7	0.061	63	39	23
3.75	1.1			72	45	27
3.80	1.0	98.9	0.077	79	49	30
3.85	0.9			91	57	33
3.90	0.8	99.1	0.097	99	62	38
3.95	0.7			114	71	43
4.00	0.7	99.2	0.122	125	78	43

This table shows the percent of molecular SO_2 present in the pH range from 3.0 to 4.0. Multiplying this percent by the free SO_2 will give the ppm (mg/L) of molecular SO_2. To attain a desired level of molecular SO_2, the amount of free SO_2 needed can be determined by dividing the desired molecular (mg/l) by the percent available at the given pH. For example, if the wine pH is 3.5 and the desired molecular level is 0.8 mg/L, then the needed amount of free SO_2 would be calculated 0.8/0.02 = 40ppm free SO_2.

Figure 18 – Overview of the percentile the three different forms of free SO_2 is present in, at given pH-values (Enartis, 2020a)

Sulfur dioxide (SO_2):

Looking at Figure 18 in appendix H, the calculation for knowing the molecular SO_2 of a sulfite addition at a given pH is as follows:

$$Free\ SO2\ added\ \times\ Percentile\ available\ molecular\ SO2\ at\ given\ pH = Molecular\ SO2\ in\ product$$

In this case, 60 mg/L of free SO_2 is added to a product at 4,47 pH. As the table only goes up to 4 pH, the percentile available molecular SO_2 at that given pH is used:

$$60\frac{mg}{L} \times\ 0{,}007 = \mathbf{0{,}42}\ \boldsymbol{\frac{mg}{L}\ molecular\ SO2}$$

Potassium metabisulfite is a white crystalline salt, which contains 57,6% sulfur dioxide (SO_2). The following equation can be used to determine how much potassium metabisulfite to add by weight to add a given amount of free SO_2 to the product: (Vinlab, 2017)

$$\frac{Volume\ (L)\ x\ free\ SO2\ addition\ (\frac{mg}{L})}{0{,}576} \div 1000 = Potassium\ metabisulfite\ (g)$$

In this experiment, 60 mg/L of free SO_2 was added to 0,44L cans:

$$\frac{0{,}44\ (L)\ x\ 60\ (\frac{mg}{L})}{0{,}576} \div 1000 \approx \boldsymbol{0{,}046\ g\ potassium\ metabisulfite}$$

Net volume of cans:

Net volume (ml) of cans were calculated by the density equation: (Gregersen, 2017)

$$\frac{Net\ weight\ (g)}{Density\ of\ the\ product\ (\frac{g}{ml})} = Net\ volume\ (ml)$$

Relative density of the product / specific gravity (g/ml): 1.014

Beer project NMR Manual

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February 2021

1 Introduction

This manual provides instructions for taking beer samples through all the steps from sample collection to preparation and NMR-acquisition. Please note that this document is not a replacement for proper training, and that none of these procedures should be performed without the appropriate HSE training.

2 Working with beer samples

When working with beer samples it is important to keep in mind that the samples are in a more or less active state of fermentation, and are quite sensitive to both contamination and change over time. Make sure to always work with proper sterile technique, and to keep samples frozen when not used. Prepared NMR-samples should be run within 24h of preparation.

3 Sample filtering

- Fill sterile syringe with beer sample and attach sterile filter.
  - Filter: 0.2 µm pore size, 25mm diameter, luer lock (female).
- Press sample through filter into appropriately sized tube, you should have at least 2 mL filtered sample. Change filter during the process if necessary.
- Note sample ID/name and date on tube.

4 Sample preparation for filtered samples

- Thaw sample (if frozen).
- Place sample in ultrasonic bath for 10 minutes (with the tube lid open) for degassing.
- Move 720 µL of the sample into an appropriately sized tube.
- Add 80 µL buffered stock solution to the same tube.
  - Buffered stock solution: 500 mM sodium phosphate pH 7.4 buffer containing D_2O (99%) and TSP (1%).
  - If there is precipitate in the buffered stock solution, warm slightly in water/hand and vortex until mixed.
- Vortex shortly to mix.
- Move 600 µL mixed sample into 5mm NMR-tube.
- Note barcode on tube lid and record this along with sample ID/name.
- Gently press the tube lid onto the NMR-tube until a click is heard.
- If bubbles are present in the tube, shake firmly to settle the sample in the bottom of the NMR-tube.
- Wipe the glass tube with clean, lint free cleaning paper to remove grease and debris that can affect the acquisition.
- Place the tube in the sample holder. Remember the placement of the different samples, this will make it easier to place in the NMR auto sampler later.

5 Software-interface overview

When operating the NMR-magnets, there are two main software interfaces you will use, these are Topspin and IconNMR. Topspin is the main software that controls the acquisition process, as well as lets us view and analyze our spectra after acquisition. Here you will not use Topspin directly to run samples, instead using the automation software IconNMR that lets us queue multiple samples with pre-made parameter settings in order to maintain the same procedure for all samples. The following figures will show an example of the interfaces of both applications with important sections annotated.

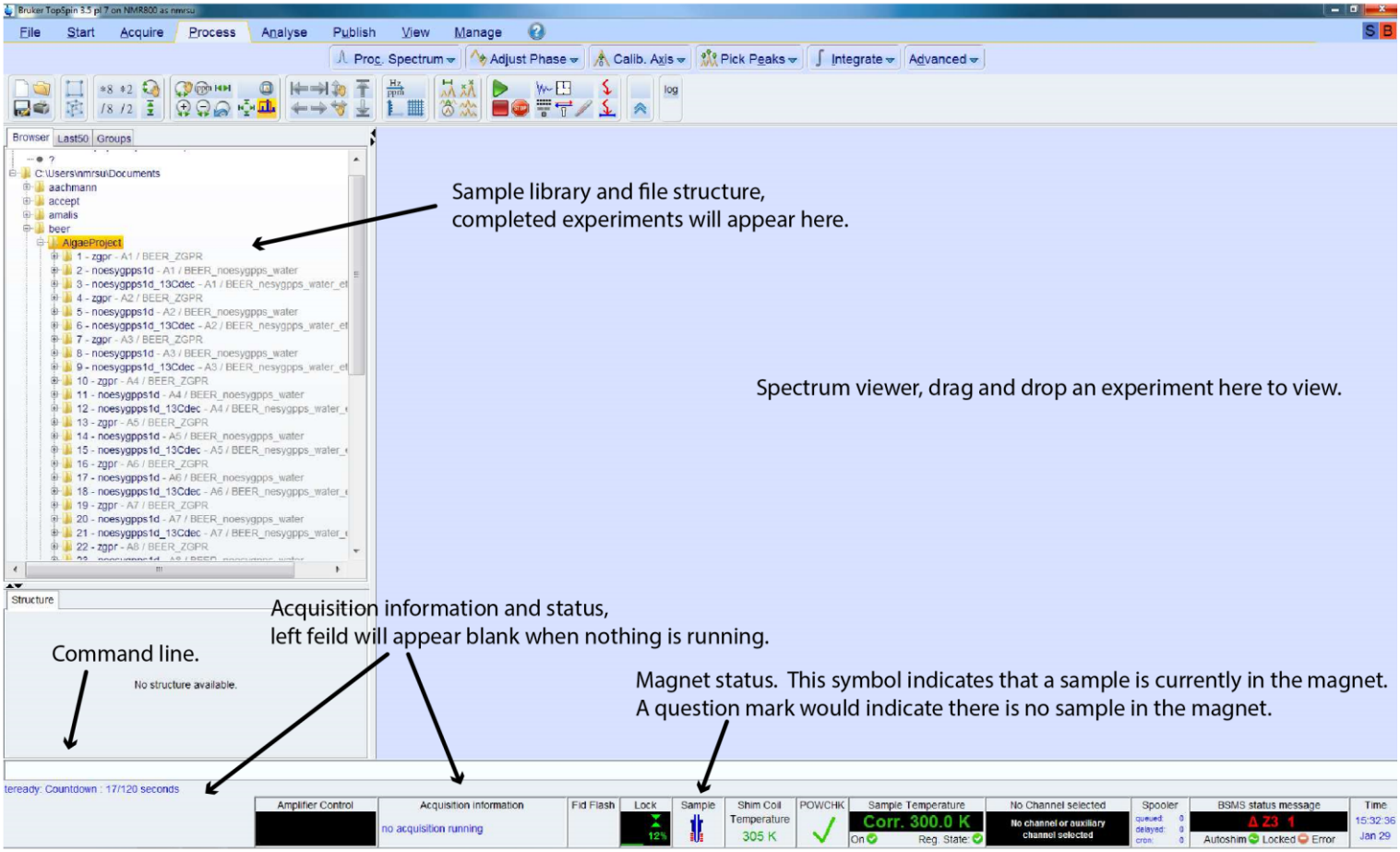


Figure 1: TopSpin interface overview.

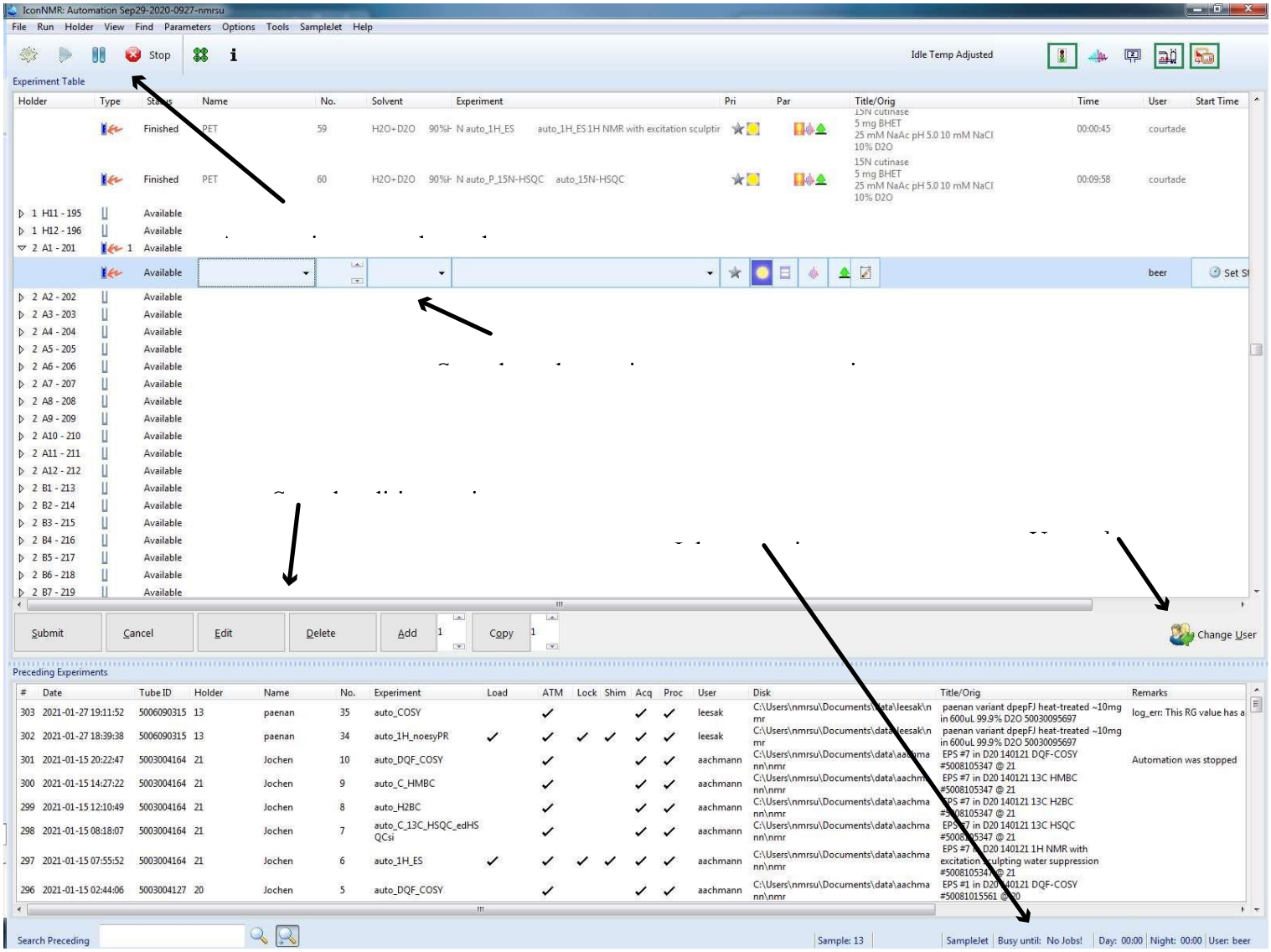


Figure 2: IconNMR interface overview.

6 NMR analysis procedure

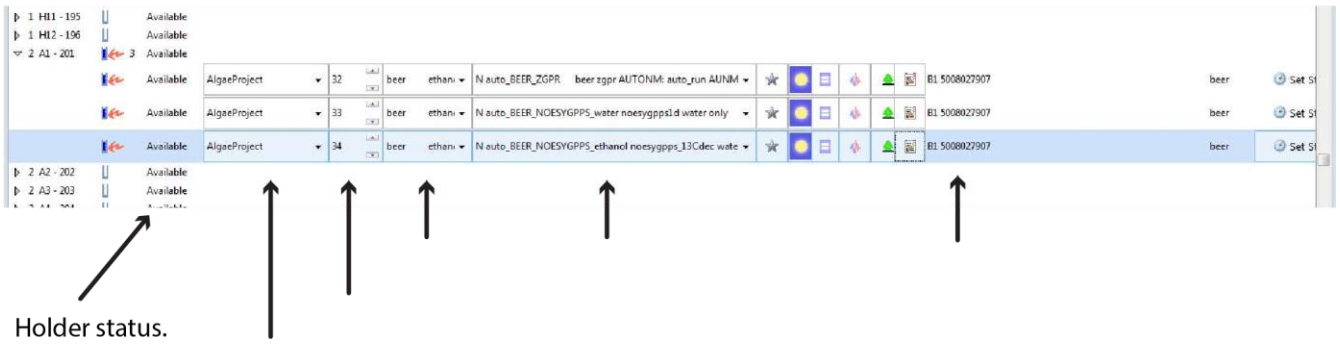
- - Check that Topspin and IconNMR is running
  - Topspin can be started through the desktop icon for Topspin.
  - IconNMR can be opened by typing "iconnmr" in the topspin command field, and selecting the "automation" option in the startup window, see figure 3.
- Check if acquisition is running in Topspin by reading the lower right-hand side of the Topspin interface, see figure 1.
- Check if automation is running in IconNMR by reading of the top left-hand side of the IconNMR interface. If the start button is active automation is off, if it's inactive automation is running.
  - If acquisition and automation is running the experiments can be set up and queued as usual, with some exceptions in the procedure for placing samples in the autosampler, noted in section 7.
  - If acquisition is running but automation is not, the experiment can be set up, but not queued.
  - If no acquisition or automation is running, automation needs to be started before the samples can be run, see section 9.
  - If no acquisition is running, but automation is, you can proceed to the next steps.

Figure 3: IconNMR startup interface.

7 Sample setup in IconNMR

- Change to the correct user, in this case the "beer" user, if you did not do this when opening IconNMR.
- Select a set of available sample holders in the IconNMR interface, preferrable in sequential order and in the same sample rack if possible.
- Make sure the holders are available by checking the physical rack.
  - If you are placing multiple samples, or replacing old ones, taking the rack out of the autosampler and into the computer room will make the next steps easier. Note that this can only be done when no acquisition or automation is running. For instructions on how to retrieve and replace the rack, see section 8 of this manual.
- Select the first sample holder in your sequence and click the add option in the lower panel. A line will appear containing four drop-down menus, as well as a few symbols, see figure 4.
    - Note: Be careful with scrolling while editing, as this will scroll through dropdown menus if hovering over one. Best practice is to always collapse a holder after editing and double check all experiments before submitting.
  - The first field indicates the directory, or folder, where the samples will be placed.
  - The second field indicates the experiment number within the directory. Each sample will create its own folder in the directory with the number specified in this field. Make sure these are unique and sequential. If you provide an experiment number that already exists in the directory the experiment will be skipped.
  - The third field indicates the solvent used in the sample. In our case you will use the "beer" preset.
  - The fourth field indicates what experiment to run. In our case you will use the "beer" presets here. There are four available, see table **??** for names and descriptions. If you are unsure of what to use, talk to your supervisor.
    - You may want to run multiple experiments on the same sample, to set this up simply press the "add" button on the lower panel while the current sample is highlighted and select a different experiment.
  - To the right of the six symbols there is a field where you can name your sample. Use sample ID or other identifier. It may also be useful to add the barcode of the NMR-tube in this field, this will appear in the "title" tab of the experiment, and can be done with the barcode scanner next to the computer.

- When a sample has been properly filled in it can be copied into the remaining sample holders using the "copy" option in the lower panel. After copying the name and barcode will need to be changed. The experiment number should increment correctly automatically. Make sure all names and experiments are correct after copying. Note that copying can only be done into sequential, empty sample holders.
- Replace the sample rack in the autosampler if applicable.



Holder status.

Solvent. Experiment parameters. Sample name.

Sample number.

Directory name.

Figure 4: Sample holder in IconNMR with four different experiments specified.

7.0.1 Experiment list and routine:

Table 1: Name, description and usage of NMR experiments

Name in IconNMR from experiment drop down list (EXP)	**Pulse program name (PULPROG)**	**Description**	**When to use**
N auto_BEER_ZGPR	zgpr	1D proton spectrum with water suppression	This is a short experiment used to determine the position of the ethanol signals. After acquisition a processing script is automatically run that picks the ethanol peaks (a quartet at 3.6 ppm and triplet at 1.2 ppm) and generates a shape file (beershape)
N auto_BEER_NOESYGPPS_water	noesygpps1d	1D proton spectrum with water suppression	This experiment suppresses only the residual water signal, the ethanol signals remain.
N auto_BEER_NOESYGPPS_ethanol	noesygpps1d_13Cdec	1D proton spectrum with water suppression, ethanol suppression, and 13C decoupling of ethanol CH2 group at 3.6 ppm	This experiment uses the shape file generated in the first experiment and suppresses the ethanol signals. There is also 13C decoupling but only for the CH2 quartet (3.6 ppm)
N auto_BEER_13C-HSQC	hsqcedetgpsisp2.3	2D 1H-13C HSQC spectrum	This experiment is not required for all samples. It is used when confirmation of identified components is required.

Routine:

Routine acquire:

1. N auto_BEER_ZGPR
2. N auto_BEER_NOESYGPPS_water
3. N auto_BEER_NOESYGPPS_ethanol

No need to run the ethanol suppression experiment for samples that contain no ethanol (e.g. control samples). If 2D HSQC spectrum is required can acquire at the same time as the fourth experiment or can acquire later. If acquired later then use:

1. N auto_BEER_NOESYGPPS_water
2. N auto_BEER_13C-HSQC

8 Autosampler

- On the 800-magnet you will find an autosampler, this mechanism will run our samples for us, we only need to place them in the right locations and give the right instructions.
- On the autosampler you will find a screen, check if the magnet is empty (indicated by two lines. A number on screen indicates a sample is running).
- Select wanted location by pressing "select rack", then the rack number. The autosampler will now rotate the appropriate rack to the small door in the sampler. If a sample is running this rotation might take a while, make sure the door is closed while rotating.
- Lift the rack out carefully and place your samples in the desired location.
  - NOTE: Do not lift the rack out if automation is running. You may still place or remove samples while the rack is in the autosampler.
  - NOTE: If automation is running you will have a time limit to place/remove samples from the rack, this will be displayed on the autosampler screen, to extend this limit press the "more time" option on the screen.
  - Remove any old samples if necessary.
- Carefully replace the rack in the correct orientation, indicated by the slanting corner.
- Reset screen by pressing "system status".

9 Submitting samples and starting automation

- When all samples are correctly entered in their sample holders they need to be submitted in IconNMR. To do this, highlight all the relevant sample holders by shift-clicking the first and last holder, and select the "submit" option in the lower panel.
- If automation is not running it must be started in IconNMR. Before you do this, make sure no acquisition is running in Topspin and that no sample is inside the magnet. Starting automation is done by selecting the "start" option in the top left-hand corner of IconNMR and selecting what sample to start on, this will be the first sample in your queue.
- Take note of run time and estimated time of completion in the bottom panel of IconNMR.

10 Removing and storing samples

After your experiments are done, and you are have made sure all the acquisitions were successfully, you should remove your samples in order to make room for others using the machines. Remove physical samples from the auto sampler and delete them from IconNMR so the sample holder becomes available. Store the samples in a freezer or other appropriate location.

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