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# **Phenols in beer derived from wood**

Qualifying and quantifying different phenols in beer aged with  
wooden chips

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## Sammendrag

Øl har blitt lagret på trefat siden produksjonen av fat startet for over 1000 år siden, og fatlagring av øl har blitt mer populært de siste årene etter mikrobryggeri-bølgen startet. Men hva skjer egentlig med øl når det fatlagres, og hvordan påvirker det smaken? Dette er et veldig stort tema, selv for en masteroppgave. Dermed fokuserer denne oppgaven spesifikt på om fenoler som er ekstrahert fra trevirke under fatlagring, og hvordan de påvirker smaken til det ferdige produktet. Dette temaet er relativt nytt innen både kjemisk- og sensorisk analyse, så det finnes lite litteratur som omhandler fenoler i fatlagret øl. Det finnes derimot mer forskning på fatlagret vin, hvor de viktigste fenolene fra trevirket som påvirker smak er vanillin, 4-vinyl-guaiacol og eugenol.

Dette startet med at øl av typen «Belgian strong ale» (11% ABV) ble lagret i glassbeholdere med treflis i 6 måneder. Treflisene kom fra fransk eik, både lett- og medium-brent, amerikansk eik, lett-brent, og ubehandlet norsk bjørk. Med blank-prøver og 2 replikaer av hver type lagring, resulterte dette i 10 typer. Prøver ble tatt ut underveis for å kartlegge utviklingen under lagring, henholdsvis etter 0, 1, 2, 4 og 6 måneder. Disse prøvene ble analysert med Folins metode for totalfenol og med HSGC-FID for å kartlegge aromatiske komponenter og andre stoffer som påvirker smaken til øl. Deretter ble typene analysert av et «semi-trent»-smakspanel bestående i all hovedsak av kolleger fra Vinmonopolet. Denne sensoriske analysen foregikk over en kveld hvor deltakerne evaluerte grunnsmaker, lukter, øvrige smaker, farge, tekstur og kompleksitet, ved hjelp av 9-punktskala og CATA. Resultatene fra 9-punktskalaene ble deretter analysert vha. PanelCheck™ for å kartlegge diskrimineringsevnen og repliseringsevnen til deltakerne. Resultatene fra CATA ble ført inn i Excel og bearbeidet. Folins metode fant at det var rundt 1150 ( $\pm$  150) mg GEA/100 mL med fenoler i de forskjellige prøvene. Konsentrasjonen i ølet hadde heller ikke endret seg nevneverdig utover de 6 månedene med lagring. HSGC-FID registrerte ingen fenoler, men heller ingen stoffer med sensoriske egenskaper som kunne forveksles med fenoler. CATA-testen viste at deltakere smakte og luktet vanilje og krydder av ølet, samt toner som minnet om ung og gammel eik. Dette indikerer at det kan være fenoler i ølet, trolig vanillin og polyfenoler. Konklusjonen ble at det trolig er fenoler i ølet som følge av fatlagring, blant annet vanillin. Grunnet problemer med metode og budsjett, så gir ikke denne oppgaven noe mer definitivt svar enn det.

## Abstract

Beer has been stored in wooden barrels since the production of barrels started over 1000 years ago, and barrel-aging beer has become more popular the later years since the microbrewing trend started. But what happens to beer when it is barrel-aged, and how does this affect the flavour? This is a very big subject, even for a master thesis. Therefore, this thesis focuses on how much phenols are extracted from wood during barrel-aging, and how this affects taste and flavour in the finished product. This subject is relatively new within both chemistry and sensory analysis, so there is little literature about phenols in barrel-aged beer. There has been done more research on barrel-aged wine, where the most impactful phenols from the wood regarding flavour is vanillin, 4-vinyl-guaiacol and eugenol.

This started with maturing a Belgian strong ale (11% ABV) in glass containers with wooden chips for 6 months. The wooden chips were made of French oak, lightly- and medium toasted, American oak, lightly toasted, and untreated Norwegian birch. With blanks and 2 replicates of each type of wood, this resulted in 10 types. The sampling was done over the duration of the maturation to map the development of phenols, specifically after 0, 1, 2, 4 and 6 months. These samples were analysed with Folin's method for total phenolic content, and with HSGC-FID to specify which phenols were present along with other substances than can affect the flavour of the beer. The types were then analysed by a "semi-trained" panel mainly consisting of colleagues from Vinmonopolet in a sensory analysis. This took place over the course of one evening, where the assessors evaluated tastes, aromas, flavours, colour, texture and complexity using a 9-point scale and CATA. The results from the 9-point scale were then analysed with PanelCheck™ to map the discrimination- and replication abilities of the assessors. The results of the CATA were processed in Excel.

Folin's method found that there was around 1150 ( $\pm$  150) mg GEA/100 mL phenolic compounds in the samples. The concentrations had not substantially changed over the 6-month maturation period either. HSGC-FID did not register any phenols, nor any compounds that has similar sensory properties to phenols. The CATA test showed that the assessors smelt and tasted vanilla and spice in the beer, along with tones of young and old oak. This indicates that there are phenols in the beer, possibly vanillin and polyphenols.

The conclusion was that there are possibly phenols in the beer because of wood-aging, vanillin, among others. Since there were problems with the method and budget, this thesis cannot give a more definitive answer than that.

## Table of contents

1. Introduction.....	1
1.1 Background.....	1
1.1.1 History and motivation .....	1
1.1.2 Phenols, flavonoids and polyphenols.....	2
1.1.3 The importance of proteins and the coagulation of proteins.....	3
1.1.4 Protein/polyphenol complex formation .....	4
1.1.5 Formation of flavour and colour complexes .....	5
1.1.6 High gravity brewing .....	6
1.2 The brewing process .....	7
1.2.1 Malting.....	7
1.2.2 Mashing.....	7
1.2.3 Boiling.....	8
1.2.4 Fermentation .....	10
1.2.5 Carbonation.....	11
1.2.6 Maturation.....	12
1.3 The aging and maturation of beers using wooden casks or chips .....	12
1.4 Sensory science and the sensory analysis of beers .....	13
1.5 Chromatography – HSGC-FID.....	16
1.6 Folin’s method.....	17
2. Method .....	18
2.1 Brewing .....	18
2.1.1 Preparation .....	18
2.1.2 Mashing.....	21
2.1.3 Boiling.....	22
2.1.4 Fermentation .....	22
2.1.5 Maturation and aging .....	22

2.1.6 Carbonation.....	24
2.2 Sensory analysis .....	24
2.2.1 Preparation and survey creation.....	24
2.2.2 Panel Recruitment.....	24
2.2.3 Standardizing and tasting.....	25
2.2.4 Processing results.....	25
2.3 Chromatography .....	26
2.3.1 HSGC-FID .....	26
2.4 Folin’s method.....	26
3. Results.....	27
3.1 Anton Paar .....	27
3.2 Sensory analysis .....	28
3.2.1 9-point scale .....	28
3.2.2 CATA-test results .....	34
3.3 Chromatography .....	37
3.3.1 HSGC-FID .....	37
3.4 Folin’s method.....	39
4. Discussion .....	42
4.1 The brewing process.....	42
4.2 The aging process .....	43
4.3 The sensory analysis.....	43
4.4 Chromatography .....	46
4.4.1 HSGC-FID .....	46
4.5 Folin’s method.....	47
4.6 Further research .....	49
4.6.1 Brewing and maturation.....	49
4.6.2 Sensory analysis.....	50

4.6.3 Chromatography .....	51
5. Conclusion .....	51
References .....	53
Appendices .....	i
Appendix A – Sensory analysis.....	i
A-1: 3-digit codes used for the sensory analysis .....	i
A-2: Survey used for sensory analysis.....	i
A-3: Summarized CATA results (Aroma) – compiled data .....	ii
A-4: Summarized CATA results (Flavour) – compiled data .....	iii
A-5: Summarized CATA results (Colour) – compiled data .....	iv
A-6: Summarized CATA results (Texture and complexity) – compiled data .....	v
A-7: PanelCheck™ statistical analysis plots .....	vi
A-8: CATA results: Aroma – Raw data.....	xvi
A-9: CATA results: Flavour – Raw data .....	xviii
A-10: CATA results: Colour – Raw data.....	xix
A-11: CATA results: Texture/Complexity – Raw data .....	xxi
A-12: 9-point scale – raw data .....	xxiv
Appendix B – Chromatography.....	xxxii
B-1: HSGC-FID – Raw data .....	xxxii
Appendix C – Folin’s method .....	xxxvi
C-1: Abs <sub>765</sub> – stock solutions – raw data .....	xxxvi
C-2: Standard curve .....	xxxvi
C-3: Abs <sub>765</sub> and converted data – samples – compiled data .....	xxxvii
C-4: Phenolic content over time – Blank – Diagram.....	xl
C-5: Phenolic content over time – Light French oak – Diagram.....	xli
C-6: Phenolic content over time – Medium French oak – Diagram.....	xlii
C-7: Phenolic content over time – Light American oak – Diagram .....	xliii

C-8: Phenolic content over time – Norwegian birch – Diagram .....	xliv
Appendix D – Miscellaneous .....	xlv
D-1: Codes for extracted samples during maturation .....	xlv
D-2: Codes for HSGC-FID .....	xlv
D-3: Brix, Plato, SG conversion table .....	xlvii

# 1. Introduction

## 1.1 Background

### 1.1.1 History and motivation

The brewing of beer is estimated to have started between 14500 and 7000 years ago, when humans first began to settle down in permanent settlements along the Fertile Crescent or other similarly fertile areas around the globe (Meusdoerffer, 2009). Whether these prehistoric individuals developed agriculture to specifically produce this alcoholic beverage, or if it was simply a coincidence, is still not clear due to insufficient archaeological evidence. What is clear is that the discovery of the fermentation process and the production of beer changed the course of human history.

The first uses of barrels in the aging process can possibly be traced back to monasteries in Europe during the middle ages. During the reign of King Edward of England, monasteries in northern England had its own brewing facility, described to have a storage room with 14 barrels of varying sizes. During the 16<sup>th</sup> century, barrels were common containers for transport and storage around Europe. The usage escalated further during the following centuries (Meusdoerffer, 2009).

Despite the 800 so years long tradition, the chemical extractions from woods and barrels used for aging beer or the sensory effects of these have not been widely researched. The objective of this thesis will be to ascertain these factors, specifically which phenols are extracted from the wood itself, how much of these are extracted, and how this effects the flavours, aromas, and the overall impression of the beer.

Some assumptions were made at the start of this project:

1. Since the phenols, flavonoids and polyphenols are quite large, they are probably not very easy to extract from the wooden chips themselves. Many of them are also hydrophobic to a certain extent. Therefore, a larger concentration of solvents will be needed.
2. The natural solvent for hydrophobic compounds in beer is ethanol. Therefore, a higher concentration of ethanol is desirable. Preferably above 10% ABV.
3. The wooden chips used in this experiment should be seeping in the beer for a longer period, preferably around 12 months but for a minimum of 6 months.
4. Since this is a food product, it should also be possible to enjoy the finished product.



5. The phenol extract should have a noticeable effect on the taste of the beer.
6. Even though wooden chips would be used, a large quantity of beer would need to be produced so that the ratio between beer and wood would be realistic.

To best accommodate these assumptions, some choices had to be made. In order to extract phenols from the wood, a beer style suited for higher concentrations of ethanol is required. The style cannot at the same time be overpowering, and therefore make the extracted phenols undetectable when consumed. To accommodate this, lighter Belgian styles are preferable to the heavy and full-bodied stouts which naturally have a lot of intense chocolate- and coffee flavours. The recipe will need to result in a beer with 10% ABV or higher, while still having balance. Regarding scale, it was decided that 100L of beer would be adequate to have the necessary volume for different kinds of wood and having parallels. This demands 2 brewing sessions since the equipment on hand only holds 50L.

#### 1.1.2 Phenols, flavonoids and polyphenols

There are many compounds that contribute flavour to beer, including esters, aldehydes and organic acids. Phenols can give the beer aromas and flavours like vanilla or other spices, or tones like smoke or burnt. The importance of phenols and polyphenols as flavouring agents in beer itself seems however to be controversial (Wannenmacher, et al., 2018). Phenols are a class of organic substances where a benzene ring that has a OH-group attached. Within this classification, molecules like flavonoids and polyphenols are also included.

Polyphenols are large molecules that consists of multiple phenol subunits. They can be classified as hydrobenzoic acids, hydroxycinnamic acids, flavonoids, stilbenes and lignans. They typically impart an astringent flavour due to their tanning activity when interacting with salivary proteins rich in proline. They can also change the bitterness and overall mouthfeel of the beer.

Flavonoids can be further classified to flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (Hardman, 2014). These are found plants and have a large spectrum of usages ranging from colours, protection, to different signalling compounds. In nature, they can be found in fruits, vegetables, grains, bark, roots, stems and flowers. By association, they can therefore be found in plant-based beverages like tea, wine or beer. They have anti-oxidative and anti-inflammatory properties among others and can therefore be of great health benefits (Panche, et al., 2016). A sub-type of the flavonoids are the proanthocyanidins, also called “condensed tannins”, which do contribute to the sensory

profile of the beer regarding their haziness by interacting with proteins (Wannenmacher, et al., 2018).

Volatile monophenols and decarboxylated phenols can impart specific flavours like spices, clove-like and vanilla along with sweetish tones if balanced correctly. If the concentrations of these are too high, the flavour might be more reminiscent of solvents or the aroma of medicinal products. These phenols are created either during the boiling process or the fermentation. When wood-aging the beer, these phenols can originate from the wood itself through degradation of hemicellulose and cellulose. They are degraded into furfural and 5-hydroxymethylfurfural, which with the release of the bound lignin can create phenolic compounds like vanillin, syringaldehyde, guaiacols, eugenol, coniferaldehyde and sinapaldehyde (Wylter, et al., 2014).

The phenolic content in beer decrease over time when aging due to degradation, like the way the bitterness in beer decreases over time. Li, Zhao, Cui, Sun and Zhao found in 2016 that the phenolic content decrease between 16% and 23% over the course of a 6-month storage period (Wannenmacher, et al., 2018).

Vanillin is a monophenol and classified as an aromatic aldehyde (Store norske leksikon, 2020). It can be produced in several different ways, be it phenylpropanoid pathway with phenylalanine in *V. planifolia* (Dixon, 2011), through the degradation of hemicellulose and cellulose mentioned above, or by POF+ (phenolic off-flavour) yeast strains (Barnes, 2020). Vanillin imparts, as the name suggests, vanilla flavour to the beer. It generally has a detection threshold in beer of approx. 40 µg/L in beer.

4-vinyl guaiacol (4VG) and eugenol are other monophenols where eugenol is derived from 4VG, and they can give beer a substantial impact in flavour. The most common tastes they impart are clove-like, peppery, smoky, spicy and/or roasted. The perception threshold of these in beer is about the same as for vanillin, that being 40 µg/L (Barnes, 2020).

### 1.1.3 The importance of proteins and the coagulation of proteins

There are other processes happening during the brewing process other than just the release of fermentable sugars and their conversion into ethanol. There are other substances that are important to the sensory profile of the beer, and proteins are one of those groups.

Proteins in beer have several different roles during the brewing process. Large protein complexes with a high molar mass ( $\geq 10^3$  kDa) improve the beers overall texture and helps

with the formation of foam. Foam is important since it makes the beer more appealing and helps release aromas. These large proteins can also be involved in haze formation, through protein/phenol interactions (further reading in section 1.1.4). Medium sized proteins ( $10^3$  Da –  $10^3$  kDa) in beer contribute by improving the stability of the foam and helps the retention of carbon dioxide. Proteins with lower molar mass ( $<10^3$  Da), peptides and loose amino acids are mainly used during the fermentation for the yeast's metabolism. These lower mass proteins can also interact with reducing sugars in Maillard reactions, forming new compounds that improve flavour and/or colour. Typical flavours that can be perceived as a result of this are biscuits, bread, nuts, caramel, dark chocolate and coffee (Skistad, et al., 2016). The Maillard reaction also creates a brown hue during the third stage of the Maillard reaction where reactive carbonyl compounds react with amino groups forming melanoidins. Melanoidins are dark-coloured, insoluble polymers containing nitrogen.

During the mashing and boiling processes, the hydrolysis of proteins is important for adequate fermentation later. A study in 2006 found that if there is not a high enough level of hydrolysis during the mashing, then there will be a larger concentration of high molecular mass proteins in the wort. These proteins would then denature and coagulate with phenols. These complexes would then precipitate to the bottom, making the nitrogen unavailable to the yeast for their metabolism. During the boiling, a plethora of different compounds are formed including reducing compounds, melanoidins and volatile heterocyclic compound through the Maillard reactions.

During the fermentation, between 40% and 70% of the free amino acids are used up by the yeast for their metabolism. If there has been insufficient hydrolysis during the mashing, then the yeast must synthesize the needed components if able. This results in the formation of waste products like vicinal diketone, including diacetyl and pentane-2,3-dione. These two compounds may result in a buttery flavour in the beer, significantly changing the flavour profile.

(dos Santos Mathias, et al., 2014)

#### 1.1.4 Protein/polyphenol complex formation

As briefly touched on in section 1.1.3., protein/polyphenol interactions can substantially affect the sensory properties of the beer.

The haziness of the beer is the result of one such interaction. Studies dating as far back 1959 have observed a clear connection between proline-rich proteins/polyphenols interactions and

haziness in beer. This was often related to a group of proline-rich proteins called hordeins which are found in barley. This was further supported when the same interaction could not be established using polypeptides that did not contain proline, whereas the addition of other proline-rich peptides and polyproline resulted in haziness. The proteins, peptides and other amino acid polymers that could achieve haziness were called “Haze-Active” proteins (HA). The haziness was also dependent on the type of phenolic compound as well. Research in the 70’s and 80’s observed that simple phenols and polyphenol monomers could not interact with HA-proteins to create haziness. The group of polyphenols naturally found in beer that could interact with HA-proteins were the proanthocyanidins and were therefore called HA polyphenols. These consisted of monomers, dimers, trimers and higher polymers of catechin, epicatechin and gallic acid.

The haziness is not the only result of protein/polyphenol interactions. As briefly mentioned in section 1.1.2, polyphenols can affect flavour as well. Tannins, sub-category of polyphenols which is present in beer, tea and wine, can cause an astringent taste when interacting with proline-rich proteins, like in human saliva. The astringent flavour might be tied to how tannins react with proteins, they precipitate into small particles which create a tactile feeling by the trigeminal nerve.

(Siebert, 1999), (Siebert, et al., 1996)

#### 1.1.5 Formation of flavour and colour complexes

While the Maillard reactions are mainly responsible for the brown hue that beer is known for (see section 1.1.3.), there are other complexes that affect the sensory properties of the beer, both regarding flavour and colour. These complexes include melanoidins, protein-polyphenol complexes and metal complexes.

Melanoidins, as explained in section 1.1.3., are protein-sugar complexes created through Maillard reactions. Melanoidins are formed during the kilning, mashing and boiling processes when reducing sugars and proteins interact (dos Santos Mathias, et al., 2014). Melanoidins impact flavour as well colour, giving the beer malty and roasty tones. The intensity of these flavours depends on the type of beer, having low intensity in light lagers and high intensity in stouts and barley wine for instance (Barnes, 2020).

Protein-polyphenol interactions between tannins and proline-rich proteins affect both the flavour and the look of the beer, as explained in section 1.1.4, by giving the beer a certain haziness and an astringent taste (Siebert, 1999). The haziness varies depending on the type of

beer, being the typical characteristic of wheat-based beers such as German weissbier and Belgian witbier.

While metal ions themselves can alter the sensory properties of the beer, like elevated levels of magnesium imparting a bitter taste, they can also form complexes with different compounds which then impact the sensory profile. Nickel ions can interact with isohumulone, the contributor of bitterness from hops, to increase foam stability dependant on the concentrations of these compounds (Luykx, 1960).

#### 1.1.6 High gravity brewing

High gravity brewing has become more and more popular over the years as the understanding of brewing techniques continues to grow and the technologies continues to improve. For a business standpoint, high gravity brewing reduces energy consumption, labour cost and equipment cost per volume of beer, while increasing brewing capacity and the yield from the raw materials.

There are however some side-effects on the beer itself when using this method. The yeast cells are increasingly more inhibited the higher the gravity of the wort is, and therefore the viability of the fermentation process decreases. This is due an increase in osmotic pressure, as well as the accumulation of “waste products” like ethanol, carbon dioxide, fatty acids and esters. The yeast is also strained due to the decreasing levels free amino acids and amino-bound nitrogen, limiting cellular growth and replication, along with decreased levels of oxygen and other nutrients for the yeast.

The increase in wort gravity also result in increasing levels of acetates and esters in the finished product. If the SG of the wort was increased from 1,042 (10,5 °P) to 1,083 (20,0 °P), then the concentration of ethyl acetate and isoamyl acetate would increase by a factor of 4, giving the beer flavours of solvents and banana, respectively (Barnes, 2020). Other acetate ester can increase by a factor between 4 and 8, dependant on the ingredients used and the sugars available.

(Olaniran, et al., 2017)

## 1.2 The brewing process

The four ingredients that are needed to brew beer are water, malted grain, hops, and yeast. This was the basis for the Purity law of 1516 in Bavaria. It stated that only water, malted barley and hops could be used when brewing beer, since yeast had not been discovered yet. Some regions rejected this law however, opting to continue using herbs, spices and fruits in their beers. While the ingredients vary for the types of beer, the main steps remain the same. These steps are malting, mashing, boiling, fermentation, carbonation, and maturation.

### 1.2.1 Malting

Malting is the process of preparing the cereals for the rest of brewing process. To extract fermentable sugars from the cereals, the starch in the grains must first be converted to less complex sugars. This is done by soaking the grains in warm water, which activates the dormant embryo and starts the germination. The embryo then releases  $\alpha$ - and  $\beta$ -amylases,  $\beta$ -glucanases and peptidases to break down the starch to simpler carbohydrates the seedling can use more efficiently. When the enzymes have broken down about 40% of the starch during the germination, the sprout will begin to grow. This is important since the yeast which will be used later in the fermentation stage cannot consume starch, and therefore cannot produce ethanol. In order stop the plant from sprouting, the grain is then dried in a kiln at 50°C - 65°C while still preserving the enzymes for the mashing (Skistad, et al., 2016).

Dependant on the temperature, different kinds of malted cereals or malts can be produced. This can give varying results for colours, flavours, and aromas, such as the dark and chocolaty black malts or the caramel malts. Different kinds of cereals will also impact the final experience of the beer. Wheat has for instance a higher protein content (11,0% - 14,0%) relative to cereals like barley (8,0% - 11,0%) and rice (7,0% - 9,0%), which gives a hazier look to the beer.

After the grains have finished kilning, the malt is partially crushed to increase the surface area and provide easier access to the fermentable sugars. The malt is now ready for the mashing step.

### 1.2.2 Mashing

The purpose of the mashing process is for the enzymes to activate to extract as much of the fermentable sugars as possible to the wort. The whole process starts with soaking the cereals in warm water for a longer period. The temperature and the duration vary depending on the style of beer, the ingredients used, the recipe, or sometimes on the desired flavour. The

mashing temperature profile may also vary dependant on the enzymic composition of the cereal.  $\beta$ -glucanase is active in the range of  $37^{\circ}\text{C} - 45^{\circ}\text{C}$ , and decreases the viscosity of the mash, making it easier to strain the wort. At  $50^{\circ}\text{C} - 55^{\circ}\text{C}$ , proteins and peptides start to break down and release amino acids useful for the yeast later.  $\beta$ -amylase has an optimum temperature range of  $60^{\circ}\text{C} - 65^{\circ}\text{C}$  and denatures at around  $70^{\circ}\text{C}$ . Here,  $\beta$ -amylase converts starch to maltose while  $\alpha$ -amylase helps by converting starch into smaller components which is easier for  $\beta$ -amylase to break down. While the temperature is between  $72^{\circ}\text{C}$  and  $75^{\circ}\text{C}$ ,  $\alpha$ -amylase breaks down the rest of the starch (Skistad, et al., 2016). The mashing process takes about 60 minutes but can be shorter or longer dependant on the factors mentioned earlier. Towards the end of the mashing process, the specific gravity (SG) is measured. This measurement is needed to determine how much fermentable sugar has been extracted and is now in the wort. If the SG is at the desired level, then the wort is lautered to remove the large cereal husks and other insoluble components.

*1.2.2.1 Original gravity (OG), Specific Gravity (SG), Final Gravity (FG), Brix, and Plato* Gravity, Brix and Plato are 3 different measurement systems designed to tell how much soluble solids there are in a water solution.

Gravity is the one based on density, or rather, the relative density compared to water. Original gravity (OG) is sometimes used as the starting gravity before the fermentation process, as in how much solids were dissolved in the beginning. Specific gravity (SG) is the term which is the most used when talking about gravity. SG is the measured gravity at any given point in time, be that at during mashing, boiling, fermentation, or after fermentation. Final gravity (FG) is the term used for the gravity at the end of the brewing process (after fermentation).

Brix ( $^{\circ}\text{Bx}$ ) is based on the concentration of sucrose dissolved in water, and it is measured in degrees. 1  $^{\circ}\text{Bx}$  or degree Brix is defined as 1g of sucrose dissolved in 100g of solution. Other solids can also be measured using this method, but this result will give an approximation (Hough, et al., 1971).

Plato ( $^{\circ}\text{P}$ ) is a refinement of the Brix scale which also examines the concentration of solubles in the wort. Where the scales differ is that Plato quantifies the concentrations of extract by weight, while Brix is based on sucrose contents alone (Oliver, 2011).

### 1.2.3 Boiling

Boiling the wort has several functions, as described in this segment.

The first reason is sterilizing the wort, i.e. removing unwanted microorganisms from the brew. Yeast- or bacterial infections from third party sources, for instance the natural culture in the building, can result in a wide array of side effects. These include, but are not limited to, ester production, acidification, and outcompeting the yeast used.

Secondly, boiling lets one add other desired compounds to the wort relatively quickly. The increased temperature makes the extraction of larger and/or less water-soluble compounds easier. These mainly include phenols, higher alcohols, larger esters, larger aldehydes, and larger organic acids. There both has been, and still is a lot of experimentation on what additions can be made during the boiling process. The most used in this regard is hops, a significant ingredient in the brewing process.

Hops contains  $\alpha$ -acids which works as a preservative in beer, giving it longevity, and adding bitterness which help balance the beer.  $\alpha$ -acids have two forms,  $\alpha$ -acid and iso- $\alpha$ -acid. The non-isomeric form is not particularly bitter, but iso- $\alpha$ -acid is. The isomerization occurs at high temperatures and makes the  $\alpha$ -acid more soluble. Iso- $\alpha$ -acid improves the longevity of the beer by being able to disrupt the functionality of the cell membranes of gram-positive bacteria. It also helps stabilize the foam, making a longer lasting head. Hops also contain a wide range of compounds that give flavour and taste to the beer. The most known of this is the distinct bitterness that beer has as a result of iso- $\alpha$ -acid, but flavours like citrus and tropical fruits are also typical as a result of citronellol and nerol, among others (Skistad, et al., 2016).

Hops are not the only type of ingredient that can be added as this stage. This varies for type of beer and can range from ingredients rich in carbohydrates to herbs and spices. In some brown ales designed for winter may have honey mixed in to give a higher % ABV, a sweeter taste, and a velvety mouthfeel. Gruit beer is a relatively unknown style of beer that discards the hops altogether in favour of using other herbs like birch leaves, sweetgale, rowan leaves and *Sambucus*.

After the wort has been boiled for the desired amount of time, the wort is then rapidly cooled down. This must happen quickly to keep the beer sterile. When the wort is cool enough to add the yeast, the wort is transferred to a fermentation tank to start fermentation.



#### 1.2.4 Fermentation

During the fermentation process the main objective is to convert fermentable sugars to ethanol using yeast. The fermentable sugars are distinguished as sucrose, fructose, glucose, maltose and maltotriose, consumed in that order.

This stage is anaerobe, with the fermentation tank sealed. A one-way valve, or fermentation lock, is located at the top of the tank, in order to let the carbon dioxide that is produced escape, while not letting new air into the tank. This stage is the second most time consuming during the brewing of beer. During the first 24 hours, the yeast cells have oxygen to spare and a lot of nutrients, so they start multiplying. The increase in the amount of yeast cells is most often three-fold. While the yeast cells break down glucose through glycolysis, they produce acetaldehyde as a waste product. The acetaldehyde is then converted into ethanol in order to supplement the yeast cell with more  $\text{NAD}^+$  which is needed for the glycolysis. There is some discussion if there are other reasons for the yeast to produce ethanol. A study from 2019 argues that the yeast cells produce ethanol to prevent a metabolic overload (Niebel, et al., 2019).

The fermentation usually goes on for 1 week minimum but more often for a month, depending on the type of yeast. The fermentation can be stopped earlier by killing (by raising the temperature over  $28^\circ\text{C}$ ), filtering or “cold crashing”<sup>1</sup> the yeast when the desired SG has been reached. The fermentation can also end by itself with the yeast becoming dormant. This happens if the oxygen has been used up and the ethanol has reached its maximum concentration, for the yeast cell then to start to flocculate.

Infusing the beer with more flavours can also be done in this stage, called dry hopping. Hops is used predominantly, hence the name, but other herbs and spices can also be used like vanilla, thyme, or juniper. Fruits and berries can also be used during this stage, but there are some issues. Firstly, these need to be heat-treated before addition due to the chance of wild yeasts and bacteria infecting the beer. Secondly, it is advised that these are added after most of the fermentation has been completed. Ripe fruits contain significant levels of pectins, which is a large group of large carbohydrates primarily used for gelling. If these were to be added during the boiling stage, it might cloud the beer, possibly making it less appealing (Skistad, et al., 2016).

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<sup>1</sup> Lower the temperature to  $2^\circ\text{C}$  -  $4^\circ\text{C}$  for the yeast to precipitate more easily.

Parts of the maturation process will begin during the fermentation, depending on the chemical and biological composition during that time. Though this will be further discussed in section 1.2.6, an example of this can be that the fermentation or the dry-hopping introducing some oxidizing agents to the beer. This can oxidize the ethanol produced by the yeast back into acetaldehyde.

After the SG has reached the desired level (FG), the now beer is technically ready for consumption. However, the beer goes through 2 or 3 more steps before its declared ready. These steps are an optional maturation step, carbonation, and finally maturation in the final container.

### 1.2.5 Carbonation

To improve the flavour profile and the flavour accessibility, the beer is carbonated. This is done in one of two ways.

First is natural carbonation, where the yeast used in the fermentation is introduced to produce more carbon dioxide. This is dependent on the brew being fully fermented before starting the carbonation. Fermentable sugars are added to each container, often regular sugar, before the container is fully sealed or capped. The remaining yeast cells in the brew will now “wake up” and produce CO<sub>2</sub>. If the beer has been matured before carbonation, the yeast cells may be dead, and new yeast may need to be added. This can also be required if the fermentation process has a long duration.

Second is direct carbonation. Here CO<sub>2</sub> is directly added to the beer by pressurizing the tank or container with pure CO<sub>2</sub>. This is a more time efficient method and is the preferred method on the industrial scale.

Carbon dioxide is important for the sensory experience because it provides three aspects. Firstly, the carbon dioxide in beer does the same job as acidity and tannins in wines, and that is cleaning the mouth and the pallet of fat and oil. Secondly, the beer can be perceived as sweeter than originally, since the fat and oil are removed from the pallet. This creates a new dimension of the culinary experience. Lastly does carbon dioxide provide a very fundamental aspect to the tasting of beer. The released gas helps carry aroma compounds, making the beer smell more and probably also appear more appetizing (Horne, et al., 2014).

### 1.2.6 Maturation

As mentioned in 1.2.4, there are two times beer can be matured during the brewing process with the first one being optional. The beer is often transferred from the fermentation tank to a new container for this process.

The optional step of usually reserved for wood or barrel aging, like the method used in this experiment. Either wood is added to the maturing beer, or the beer is added to a barrel. This will be further discussed in section 1.3.

The maturation step after carbonation sees the beer stored in the container it will be served from over an extended period of time, be it can, bottle, or keg. This period helps the beer develop some more nuances to the flavour, as the different compounds slowly react with each other or the environment inside the container. Other than the release of amino-acids, peptides, phenolic compounds and phosphates, among others, the physical properties surrounding the maturation can influence the development of textures, aromas, tastes and flavours. These include the ambient temperature during the maturation and the duration of said maturation, but also the shape, geometry, capacity and composition of the maturation containers themselves (Masschelein, 1986).

During “warm maturation”, which is most common in ales, the priming sugar still present in the beer will be quickly metabolised. The “green” flavours like citrus, grass and tropical fruits also gradually fade, dependant the type of beer (Masschelein, 1986). Compounds such as phenols and trans-iso- $\alpha$ -acids degrade over the maturation period, changing the flavour profile in the beer (Wannenmacher, et al., 2018). Ethanol can start oxidizing while the beer is maturing, turning the ethanol pack into acetaldehyde which gives the beer certain sensory properties that might not be desirable in the finished product (Barnes, 2020).

### 1.3 The aging and maturation of beers using wooden casks or chips

As mentioned in section 1.1.1, wooden casks have been used to store and somewhat mature beer for a long time. In later years, wooden casks and chips has been used to impart flavours to the brew that does not occur by “normal” brewing” practices. The resulting flavours and aromas vary on the type of wood used and on the degree of toasting that wood underwent.

Regrading types of wood, oak is the most common and the most favoured by the industry when using casks, specifically European and American oaks. Oak can bend enough to produce the casks, and it is also strong enough to stand transportation. Oak is also water resistant if cut and processed correctly. Wooden chips are used as mentioned earlier. Here, the

type of wood does not matter that much, but the wood must have the structural integrity to not splinter too much during the maturation (Skistad, et al., 2016). Different types of wood can impart different flavours, with spicy flavours being the most common. Cherry can for instance impart a wider variety of volatile compounds than oak, 37 volatile compounds versus 24 volatile compounds, respectively. The volatile compounds in cherry are however generally at lower concentrations than in oak (Setzer, 2016).

The degree of toasting can also impact the flavour profile of the beer in question. The lighter toasts typically impart fruitier tones with some vanilla, while darker roasts contribute more tannins, nuts, bread, spices and/or vanilla to the flavour profile (Oak Add Ins, 2020).

The reason for these flavours is often due to lactones, volatile phenols and/or phenolic aldehydes like eugenol, guaiacols and vanillin. Lactones and eugenol can stem from wood that is not heat-treated. Heat-treating the wood helps degrade hemicellulose down to vanillin, 4VG and other furan products through the Maillard reaction (Sterckx, et al., 2012). In addition to the heat-treatment, factors like pH, ethanol content and yeast strain can influence the efficiency of the extraction (Sterckx, et al., 2012).

#### 1.4 Sensory science and the sensory analysis of beers

Sensory science is a part of food science where one studies the human responses when consuming and tasting food products, while minimizing the biases that can influence this response. These influences can be branding, loyalty, advertisement or other information sources. The human response is in this instance linked to the sensory properties of the food product in question. These include aroma, taste and flavour, among others. Aroma is related to smell of a product. Taste is the collective term for the 5 fundamental tastes, sweet, sour, bitter, salty and umami. Flavour describes all the over “tastes” that can be experienced, such as apples, citrus, chocolate and more. The reason to conduct such studies is to accurately establish the sensory properties of a product, which can be used by companies, food scientists and other interested parties in order to improve the product or production in some way.

Sensory science can also be used to describe a product, which can be used to strengthen the finding in other parts of an experiment.

There are three classes of sensory analysis, these being “Discrimination”, “Descriptive” and “Affective”. The first two are analytic in nature, which is generally done by a trained panel. A trained panel consists of experts in sensory analysis, have a good repeatability, and generally have a wide repertoire of flavours and aromas they recognize. They do however remain

objective regarding how they like the product. The last one is hedonic, i.e. how well the product is liked. General consumers are mainly used for this method.

Discrimination tests evaluate how different a set of products are from one another and is qualifying in nature. The setups for the test might vary, but the objective for the panelist stays the same; “Which one/ones are different from the rest?”. The simplest form this can take is in a triangle test. Here, each panelist is served 3 samples, and asked to pick the one that is different. The trick here is that there are two products, A and B, served in sets of 3. The sets of products are repeated for each panelist and the order of the products are randomized for each serving. For instance, the first serving can be AAB and the second can be BAB. This kind of test is mainly run to improve an existing product, where one is the old product and the other is the new prototype. An example can be that a company is trying to make a yoghurt that have less fat but tastes the same. The goal in this instance is that there is no difference between the products. The important ability here for the panelist is the ability to discriminate between two products that are inherently similar. This is also important in descriptive testing where “In what way is the product different?” is also important.

Descriptive analysis evaluates the specific sensory properties and/or the perceived intensities of sensory properties in a product or products. This method is quantifying in nature. The panelists are asked to describe what tastes, aromas, flavours etc. they can perceive in a sample, and the intensity of those attributes. There are two methods for descriptive analysis that will be used in this experiment, these being “9-point scale” and “CATA”.

The 9-point scale method asks the panelists to evaluate the intensity of a sensory attribute on a scale with 9 increments, where the more intense the attribute is, the higher it ranks on the scale.

CATA (Check All That Apply) is a method used to determine the aroma- and flavour profile of a sample. The panelists are asked to evaluate the sample through smell and/or taste, and “check” all if any of the sensory properties that they can perceive. This can be difficult, dependant on how precise the sensory profile needs to be. “Does the sample taste of lemon or lime?” for instance.

Descriptive analysis demands that both the panelists and the administrator of the test have a large vocabulary regarding sensory properties. In order to develop the vocabulary needed for descriptive analysis, the administrator and the panelists generally have a meeting where they

talk through which attributes are relevant. It is also common that they taste a product like the product of the actual test.

The affective test can also be called consumer liking and is hedonic. It therefore evaluates how the consumers like the product, the prize, the packaging etc. This is typically used before launching a product on the market to evaluate how successful it may become. These kinds of tests are therefore not used in scientific research experimentation as these results would not be relevant to the sensory properties nor the chemical build-up.

The results of the 9-point scale descriptive analysis can then be analysed in a software called PanelCheck™ created by Nofima Mat (Ås, Norway). This software statistically analyses the data and presents them in diagrams and infographics that visualize the data in a more comprehensive fashion. It can run p\*MSE plots, Tucker1 plots and correlation loadings, and 2-way ANOVA, among others.

p\*MSE plots is used to evaluate the performance of the panel as well as the method. Figure 1 is a visual representation of this. The plot is divided into 4 quadrants, with quadrant I in the bottom-left corner, quadrant II in the lower-right, quadrant III in the upper-left, and quadrant IV in the upper-right. Each quadrant indicates how well the panelists perform regarding repeatability, discrimination or both. If the assessors are in quadrant I, then they have both good repeatability. If they are in quadrant II, then they have poor repeatability. Quadrant III represents poor discrimination between the samples. If the assessors are in quadrant IV, they perform poorly in repeatability and in discrimination (Lawless & Heymann, 2010).

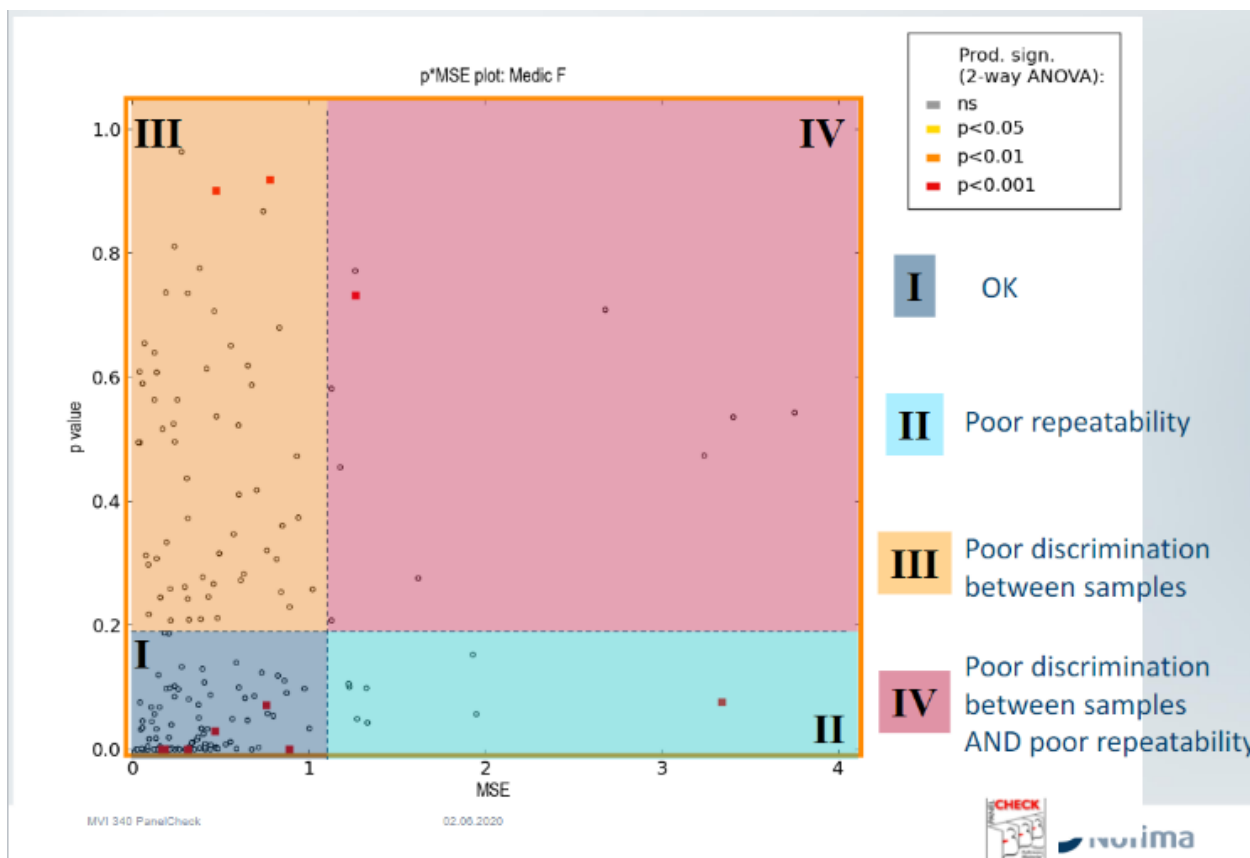


Figure 1:  $p$ \*MSE plot quadrant divisions. The different quadrants give an indication on the performance of the panelists. (Varela-Tomasco & Lengard Almlí, 2020)

Tucker1 plots consists of two plots, common scores and correlation loadings. The common scores plot the different samples. The closer these plots are, the more similar these products are. Correlation loadings how much the assessors agree with each other regarding certain attributes. The same principle applies here as with the common scores, the closer they are, the more they agree.

2-way ANOVA can be used to check the assessor effect, the product effect, or the assessor\*product interaction. The assessor effect evaluates if the assessors used the scale differently to grade the samples. The product effect assesses if there are any differences between the products regarding the different attributes. The assessor\*product interaction evaluates if the grading of an attribute is dependent on the product or on the assessor.

### 1.5 Chromatography – HSGC-FID

Gas chromatography is a separation technique that uses a gas as the mobile phase that carries the analyte through the column. The separation happens in the column before the components reaches the detector at the end. The mobile phase or carrier gas is inert, and can either be helium, hydrogen, nitrogen, argon or carbon dioxide dependant on the analyte. The carrier gas

must not interfere with the detection either, with helium or nitrogen being the most common choices. The column uses a stationary phase that can either be solid (GSC) or liquid (GLC), where gas-liquid chromatography is most common. The stationary phase in the column retains the components to different extents dependant on the molecule's affinity for the column, and thus achieving separation. The molecules diffuse back into the mobile phase at their respective rates and are carried through to the detector which analysis them.

Headspace gas chromatography (HSGC) refers to the headspace sampling technique where only the vapour above the liquid solution or the solid sample is sampled. This method only works for analytes that are volatile. The HS sampling can be either static or dynamic. Static HS sampling means that the analyte is sampled directly after the thermostated sample has reached equilibrium. Dynamic HS sampling actively extracts the volatiles from the samples using methods like “purge and trap” or “cryogenic focusing”.

The detector identifies the different compounds in the analyte after they have been separated in the column. There are a large variety of detectors, with two of the most common being a flame ionization detector (FID) and mass spectrometry detector (MS). The FID is a detector that burns the samples, producing ions of the components. These ions create a current, which are then amplified and read by a computer system. FID is widely regarded to by the best for the analysis of organic compounds due to its high sensitivity. The MS also works by ionizing the analyte before analysing it. Like the FID, MS can also be used for both qualitative and quantitative analysis. Each of them has some advantages over the other. One advantage the MS has over FID is that it is easier to identify substances that have not yet catalogued in the library. This is due to it giving out the mass spectrum of the analyte (Ekeberg, 2019) (Miller, 2005).

This experiment used helium as the carrier gas, a static HS autosampler, CP-SIL 5CB GC column and FID. This column is made of PDMS, a nonpolar silicon polymer, which separates components based on boiling points (Dysvik, et al., 2020).

## 1.6 Folin's method

(Lindon, et al., 2017)

Folin's method or the Folin-Ciocalteu assay is a widely used method to analyse the phenolic content of a food product. The method uses the Folin-Ciocalteu reagent, made of phosphomolybdate and phophotungstate, which reacts with phenolic compounds or other reducing compounds. This leads to the creation of molybdenum-tungsten blue. This hue can



be measured spectrophotometrically at around 760 nm. The results are given as mg gallic acid equivalence per 100mL (mg GAE/100mL).

## 2. Method

### 2.1 Brewing

#### 2.1.1 Preparation

The recipe used in this experiment was based on a Belgian blonde from the brewery Nøgne Ø (Horne & Eick, 2013), but was tweaked substantially. The recipe was finalized in a software called “BeerSmith 2”. This software can add the different ingredients, adjust quantities, and get an estimation on the properties of the finished beer. If the calculated properties are not right for the desired product, then gravity, bitterness and colour can be adjusted. This in turn increases or decreases the calculated quantities of barley and/or hops. There are some limitations in this program, for example how the brewing system used in this experiment was not on the list of equipment one could choose. Rather than program that in all the parameters for that specific rig, a 20L “pot and cooler” system was chosen as a stand-in for the actual system which was 50L.

In the original recipe, they used 3900 g Pilsner malt, 780 g of wheat malt and 260 g of Cara (20 EBC). This would give a SG of 1,044 which was deemed far too low for this experiment. They also used the hops Aurora (10% AA), Bobek (5% AA), Cascade (6% AA) and Saaz (4%).

There were a lot of malt in stock at NMBU, so the recipe was adjusted in order to use the available malts instead of those in the original recipe. The selected malted barleys were Pilsner, Wheat and CaraRed from Germany (GER, 40 EBC). The SG were calculated and adjusted to 1,091 when the values were entered in “BeerSmith 2”. The bitterness was adjusted to 22,0 IBU, and the colour to 11,1 EBC. The quantities were then converted from “per 20L” to “per 50L” by multiplying them by 2,5.

Table 1: Calculated quantities of ingredients per 50L

Type	Name	Quantity	Time	Comment
Malted barley	Pilsner (Weyermann, 4 EBC)	13925 g	55 °C for 5 min 65°C for 65 min 77°C for 5 min (Mashing)	
	Wheat (Weyermann, 4 EBC)	2775 g		
	CaraRed (Weyermann , 40 EBC)	600 g		
Hop	Aurora (6,4% AA)	92,2 g	90 min (Boiling)	Bitter hop
Hop	Bobek (5,0% AA)	87,5 g	0 min (Boiling)	Aroma hop
Hop	Cascade (6,0%)	87,5 g	0 min (Boiling)	Aroma hop
Hop	Saaz (5,0% AA)	87,5 g	0 min (Boiling)	Aroma hop
Yeast	WLP545 Belgian Strong Ale Yeast	3,0 pk (approx. 100 million cells per pack, with a viability of 78- 85%, (Bryggselev.no, 2020))	1 month (Fermentation)	

Before the brewing itself could start, the yeast needed a head start in order to not be strained during the fermentation process. Fresh yeast as used in this experiment (WLP545) can be

strained easily due to potentially high gravity but is still better suited than dried yeast since the potential is so high. A culture of water and malt extract was mixed to create a wort with an SG of about 1,050. 1L culture was poured into each of 3 Erlenmeyer flasks, followed by 1 packet of yeast in each flask. The flasks were then incubated for 48 hours until the worts were ready for fermentation.

The fermentation containers were 20L plastic containers while the maturation containers were 10L glass bottles. Therefore, the wort was split into 5 equal fermentation containers with 10L of wort in each. These were further filled with an additional 10L after the second session. After the beer was finished fermenting, each container was split to 10L each.

All the containers were coded according to the wood they would be added. The codes are described in table 3.

*Table 2: Codes for the fermentation- and maturation containers*

<b>Wood type</b>	<b>Fermentation code</b>	<b>Maturation code</b>
Blank, first replicate	B	B1
Blank, second replicate	B	B2
Lightly toasted French oak, first replicate	LF	LF1
Lightly toasted French oak, second replicate	LF	LF2
Medium toasted French oak, first replicate	MF	MF1
Medium toasted French oak, second replicate	MF	MF2
Lightly toasted American oak, first replicate	LA	LA1
Lightly toasted American oak, second replicate	LA	LA2
Untreated Norwegian birch, first replicate	NB	NB1
Untreated Norwegian birch, second replicate	NB	NB2

For the carbonation to not overpower the beer for later sensory analysis, the carbonation level should be 1,9. To carbonate the beer to level, 41,5g of refined sugars are needed per 10L of beer.

When it came to determine the amount of wooden chips to use for the maturation process, there were, as mentioned in section 1.2.6, no set amount of wooden chips for aging beer. To ensure that a substantial amount of phenols got extracted from the wood, it was decided to use 38g of wooden chips per 10L of beer. This is about 6 times the average recommended dosage. The wooden chips were weighed in as dry weight, and then poured into their own dry-hopping bag. The weigh-ins are in table 3.

*Table 3: The measured dry weight of the different types of wooden chips.*

<b>Type of wood</b>	<b>Dry weight (g)</b>
LF1	38,6
LF2	38,7
MF1	38,6
MF2	38,8
LA1	38,7
LA2	38,6
NB1	38,4
NB2	38,1

### 2.1.2 Mashing

The mashing process followed the description in section 1.2.2 in accordance with the durations and temperatures mentioned in tables 1.

Because of the target of 100L of finished beer, two sessions were required. The weigh-ins of malted barley are in table 4.

Towards the end of the mashing of both sessions, the SG has measured using digital refractometers. These measure in °Brix, which can then be converted into SG through tables (Appendix D-3). At the end of the first session, the SG read out to be 1,084, a bit lower than calculated. During the second session, there were some unforeseen circumstances. Towards the end of the mashing process, the SG was measured to be about 1,070. Since this was a bit lower for the calculations, additional measurements were done with digital refractometers,

analog refractometers and hydrometers. Because of this, malt extract and refined sugar was added in doses to the wort to compensate. Measurements were made between the extra dosages and the readings varied from 1,050 to 1,100, jumping excessively back and forth. Most of the readings were however in the lower part of the range. This resulted in adding 2,0 kg of malt extract and 1,0 kg of refined sugar to the second batch, theoretically increasing the SG of the second batch by 1,020 and the ABV by 2,4%. Measurements were made between the dosages with the same variation in SG as earlier, making the measured SG inconsequential. This was fed back into “BeerSmith 2”, giving a theoretical SG of 1,112 when adjusting for the larger volume.

### 2.1.3 Boiling

The boiling went according to the description in section 1.2.3, with the duration and amounts as specified in tables 1 and 2 for both sessions. The timer started when the Aurora hop was added to the wort. The rest of the hops, Bobek, Cascade and Saaz, were added after 90 min boiling. The wort was then quickly cooled down, for then to be filtered and poured into fermentation containers.

### 2.1.4 Fermentation

After the wort had cooled down, the yeast was mixed to ensure an even distribution of yeast along all the fermentation containers. The yeast was added in an even amount to 5 fermentation containers. After the first session was completed, the wort was distributed into the fermentation containers, with 10L in each. The fermentation started whilst the second session took place. When the second session was finished, the remaining 10L of wort were added to the fermentation tanks the next day. Each container was marked with a code in accordance with table 3. The fermentation took place at room temperature with fermentation locks.

### 2.1.5 Maturation and aging

After the fermentation was finished after 28 days, the beer was split from the 5 fermentation containers to 10 glass maturation containers with 10L each. These were then marked the distinguished codes.

Some of the chips used in this experiment were given from a French company called “Oak Add Ins” that operates in Ludon-Mèdoc. This company specializes in refurbishing old casks used in wine production, and then selling them for usage in the production of beer, wine or spirits. The package of wooden chips contained “French oak – Lightly toasted”, “French oak

– Medium toasted” and “American oak – Lightly toasted” among others. These were chosen to be the add-ins for this experiment. The oak used to make these oak chips were first seasoned for 24 months in the open air. There is however no public information on how they exactly treat the wood and chips before they are ready for sale (Nadalié Oak Add Ins, 2020). Untreated Norwegian birch was also added to the list on account of being a type of wood that is not widely used for the maturation of alcoholic beverages. The chips of the Norwegian birch were chipped off from locally acquired firewood.

The wooden chips were then placed into “dry-hopping bags”, and then heat treated in a 1 min boil to remove any microorganisms that could ruin the beer. 2 bags with LF, 2 bags with MF, 2 bags with LA and 2 bags with NB. The bags with the chips were placed into their respective container, and then stored at 14°C for 6 months. The glass containers are depicted in figure 1, showing 8 20L containers (containing LF1/2, MF1/2, LA1/2, and NB1/2) and 2 15L containers (containing B1/2). All containers were marked with their respective code.



*Figure 2: Storage of the glass containers with the maturing beer. Each of them was sealed with a rubber cap and a fermentation lock.*

In order to analyse the development of phenols over time, 3 x 50ml beer was taken out of each container at planned intervals. The samples were taken at 0 months, 1 month, 2 months, 4 months and 6 months of maturation. These samples were then frozen for analysis later.

After the beers had matured for 6 months, an “Anton Paar” analysis was run to determine the %ABV and SG. This was to check if the samples were relatively similar in these regards,

and if all had stayed at the desired %ABV. The beers were then carbonated to finalize the brewing process.

#### 2.1.6 Carbonation

Since the beer has matured for 6 months, it stands to assume that the yeast cells are dead. Therefore, another batch of yeast was made using WLP715 which is more tolerant to ethanol than WLP545. The yeast was mixed in each maturation container along with 41,5g of refined sugars per 10L of wort.

## 2.2 Sensory analysis

### 2.2.1 Preparation and survey creation

Using an untrained panel was the only option for this project, so the preparations and the survey had to be structured around that fact. The goal was to still use a “semi-trained panel”, i.e. recruit people with some experience from tasting and/or with a genuine interest for beer and brewing. The size of the panel was deemed to be best at approx. 9 people, because of the false security in number when conducting sensory analysis (Lawless & Heymann, 2010). However, this type of panel is not suited for large scale descriptive analysis, since this format was entirely new to them.

Therefore, the best compromise was to choose the following methods, 9-point scale for tastes (sweetness, bitterness etc.) and amount of perceived “barrel-aging”, and a CATA-test (Check All That Apply) for the aromas, flavours, texture and complexity, and colour. These methods can be used with both untrained panelists/consumers and with trained panelists to certain extents. The survey was made in GoogleForms™ (see appendix A-2, Attachment 1).

In order to help the panelists with their vocabulary, my supervisor and I had a tasting of the uncarbonated beer. We wrote down all the flavours and aromas we could distinguish between in order to make a vocabulary. The vocabulary was supplemented with flavours and aromas that are common in this type of beer.

In order to not discourage the panelists, the tasting session was scheduled to be held in a meeting room instead of using the actual room on campus for sensory analysis.

### 2.2.2 Panel Recruitment

The recruitment of panelists was mostly based among my colleagues in Vinmonopolet through Vinmonopolet’s communication channels on Workplace™ in “District 10” which spans mainly the area east of Oslofjorden. Here there were a plethora of experienced tasters of

different types of alcohol. The notice was open to all that were interested and could spare the time for the sensory analysis, though it did emphasize on preferring people with experience tasting beer. The notice also opened for relatives, friends and acquaintances of the employees. The recruitment process resulted in 8 people, of these 2 women and 7 men, with 6 from Vinmonopolet, 2 partners of 2 of the employees, and 1 person working in a beer-importing company. In order to fill up the vacant slot on the panel, recruitment ensued in the student body. This was specifically in the course MVI276, “Beer brewing”, and the last panelist was recruited here.

This resulted in a group of assessors of 9 people, 7 men and 2 women, ranging from 22 to 61 years of age.

### 2.2.3 Standardizing and tasting

Before the panelists could taste the samples from this project, they first needed to be calibrated or “standardized” by tasting similar products, i.e. other beers that had been barrel aged. For this, three beers were chosen: Birrificio Il Mastio Drum Barrel (Articlenr. 5408202 at Vinmonopolet), Burning Sky Saison de Fête Barrel aged (Articlenr. 10686901 at Vinmonopolet), and Nevel Bloei (Articlenr. 11272801 at Vinmonopolet). These beers were readily available through Vinmonopolet and were to some degree like the samples, since these are all lighter ales.

### 2.2.4 Processing results

The datasets were first split into 5 parts: 9-point scales, aromas, flavours, complexities, and colour.

The 9-point scales were transferred to a spreadsheet and restructured in a format that can be analysed using the PanelCheck™ software.

The 4 other parts were restructured and compiled into spreadsheets by counting the checks in CATA as “votes”. Then these “votes” were summarized for each parallel, for instance all assessor data for LF2. After which all the parallels were compiled into spreadsheets, based on the category of data, these being aromas, flavours, colour and texture and complexity. The final datasets were then visualized as bar charts (figures 8 and 9, also in appendix A-3 – A-6).



## 2.3 Chromatography

### 2.3.1 HSGC-FID

This method is devised and described by (Dysvik, et al., 2020). The codes used for the HSGC is in appendix D-2.

The samples were first filtered with “folding filters” to remove any CO<sub>2</sub> and any large particles into Erlenmeyer flasks. 15,0 mL of the filtrate was taken out of the flasks and put into centrifugation tubes. The samples were then centrifugated at 3000 rpm for 20 min at 4oC. This results in a sedimentation of yeast cells and other particles that were not desirable for the HSGC-FID. After centrifuging the filtered samples, 10,00 g was weighed in into headspace bottles. The bottles were then sealed using Teflon-covered septas with an aluminium ring. The samples were then frozen until the gas chromatographer was available for this project.

When the gas chromatographer was made available the samples were taken out of the freezer and thawed by a senior engineer, who carried out the rest of the analysis according to the method. The standards used were acetaldehyde, diacetyl, ethylacetate, 2-butanon, 2-hexanol, 2-methyl-butanal, 2-methyl-1-butanol, 2-methyl-1-propanal, 3-methyl-butanal, 3-methyl-1-butanol, 2-methyl-1-propanol, isobutyl acetate, hexanal, isoamyl acetate, ethyl hexanoate, 3-carene, R-(+)-limonene, ethyl heptanoate, ethyl octanoate, β-citronellol, ethyl nonanoate, ethyl decanoate, phenylethyl alcohol (Sigma-Aldrich), acetoin, acetone, ethanol, 1-butanol, 1-propanol, 2- butanol, dimethylsulfide, and 2.3-pentadion (Dysvik, et al., 2020).

## 2.4 Folin’s method

To evaluate the total phenolic content in the different variants, Folin’s method was used. This determines a total phenolic content equivalent distinguished as mg GAE (gallic acid equivalence)/100mL.

First a stock solution with a concentration of 1g/L gallic acid was made. This was then diluted to make solutions of 25 mg/L, 50gm/L, 100 mg/L, 150 mg/L, and 200 mg/L. These solutions were the basis for the standard curve. 0,5 ml of each solution were taken out to be mixed with 2,5 ml 10% Folin-Ciocalteu reagent (dissolved in water) and 2,0 ml 7,5% Na<sub>2</sub>CO<sub>3</sub> buffer. After mixing, the samples were incubated at room temperature for 60 minutes. The solutions were the then poured in plastic cuvettes and were measured at Abs<sub>765</sub> with a spectrophotometer (Genesys 50, UV-Visible Spectrophotometer, thermoscientific) with 3

replicates. The measured absorbances were then written down in a spreadsheet. The averages of the measurements were used to plot out the standard curve.

In order to ensure that the actual beer samples were not measured to be outside the linear area of the standard curve, these samples were diluted to 1:20. After this was done, the diluted beer underwent the same procedure as the stock solutions, and measured at Abs<sub>765</sub>. The measurements were then written down in a second spreadsheet where they could be converted back to mg GAE/100mL in the original samples. These conversions were then plotted into their own graphs with their replicate to better illustrate the development and changes of the phenolic content over time. All the datapoints were plotted together to create a general overview of the development. The datapoints used for plotting were the converted average values for the original samples.

### 3. Results

The samples were coded with 1-2 letters and a 3-digit code. The letters represented the wooden chips used during the maturation, as described in Appendix D. The first digit being which of the 2 parallels it was from. The second being which month it was taken out, either 1, 2, 4, or 6. The third digit represented which replicate it was. The only exception was the sample from 0 months of maturation, since this would theoretically be the same across all types. These 2 replicates had the code “B00X”, dependant on the replicate. “B” being “blank”, the first “0” representing that this were taken out right when maturation started, the second “0” being “0 months”, and finally “X” being either 1 or 2.

#### 3.1 Anton Paar

The Anton Paar shows that all the samples have stayed at a relatively similar level regarding % ABV and SG, which are the two most important parameters for this analysis. The average % ABV was 10,94% ABV, with a standard deviation of 0,0746, was within the range necessary to extract the phenols from the wooden chips. The SG further supports this, indicating that the beer has fermented completely. The EBC values are similar when comparing the replicate within the types (B, LF, MF etc.). There is however a relatively large discrepancy between LA161 and LA261.

Table 4: Anton Paar results for the samples undergone 6 months of maturation, taken before carbonation. Results include %ABV (% v/v), density/SG ( $g/cm^3$ ), EBC, and energy content (kJ/100 mL).

Sample Name	Alcohol (% v/v)	Density	Colour Value	Calories (kJ/100 mL)
-	%v/v	$g/cm^3$	EBC	kJ/100ml
NB161	10,85	1,00170	21,80	317,45
NB261	10,96	1,00187	21,54	320,45
LA161	10,98	1,00176	19,68	321,31
LA261	10,93	1,00179	23,63	319,97
LF161	10,93	1,00171	23,29	319,56
LF261	10,88	1,00170	22,84	318,23
MF161	10,86	1,00171	25,14	317,90
MF261	10,91	1,00181	22,82	319,61
B161	11,05	1,00233	18,07	324,19
B261	11,07	1,00224	17,27	324,50

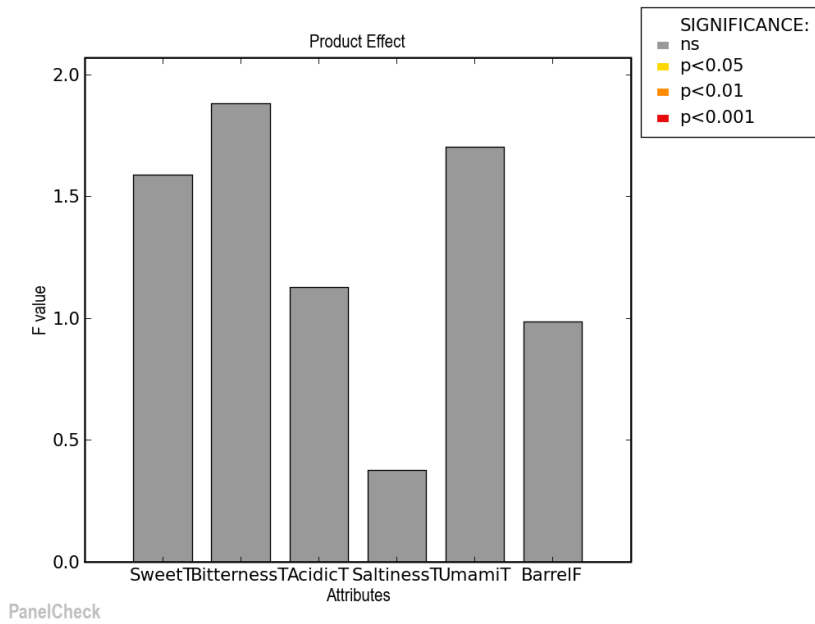
### 3.2 Sensory analysis

The results can be viewed in their entirety in Appendix A (A-1 – A-12).

#### 3.2.1 9-point scale

The raw data from the 9-point scale results were analysed through the statistical software PanelCheck in order to determine if there were any significant differences between the products, or if there were problems with the panellists' understanding of the attributes or their ability to discriminate between the samples.

The first result is the Product effect achieved through use of a 2-way ANOVA. The product effect gives an indication on how well the panel can discriminate between the samples. As seen in figure 2, the panel could not detect any significant difference between the samples regarding the chosen attributes for this method.



*Figure 3: The PanelCheck result of the product effect regarding the different attributes. There was no significant difference between the products.*

The Assessor effect gives an indication on how the panelists use the scale, and if it differs for certain attributes. This was also run through a 2-way ANOVA. The results displayed in figure 3 shows that there were significant differences in how the panelists used the scale across all the attributes, with all having a p-value less than 0,001. The F-value gives an indication that this was most apparent with saltiness and umami.

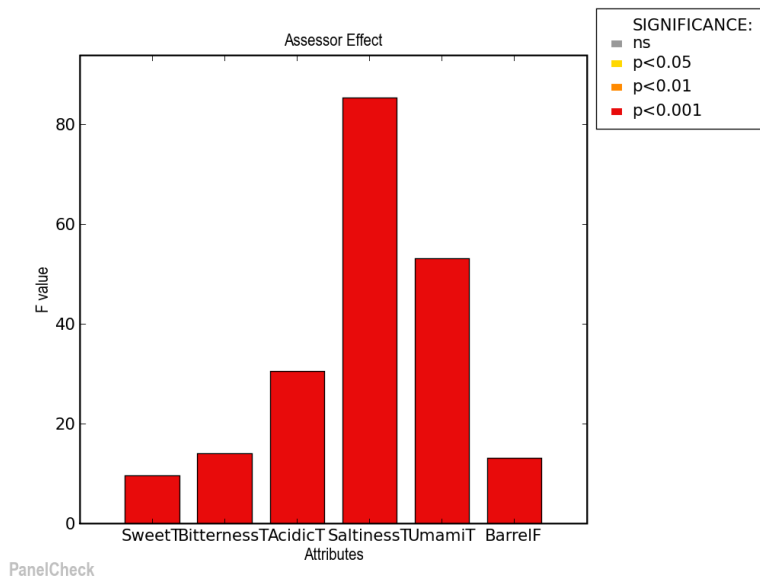


Figure 4: The Assessor effect, i.e. how the scale is used differently between the assessors. The assessors used the scale differently for all the attributes.

The Assessor\*Product Interaction estimates whether the assessors perceived the products the same way for the different attributes. Figure 4 shows that there was no significant difference, and therefore the samples were statistically perceived the same by all the assessors.

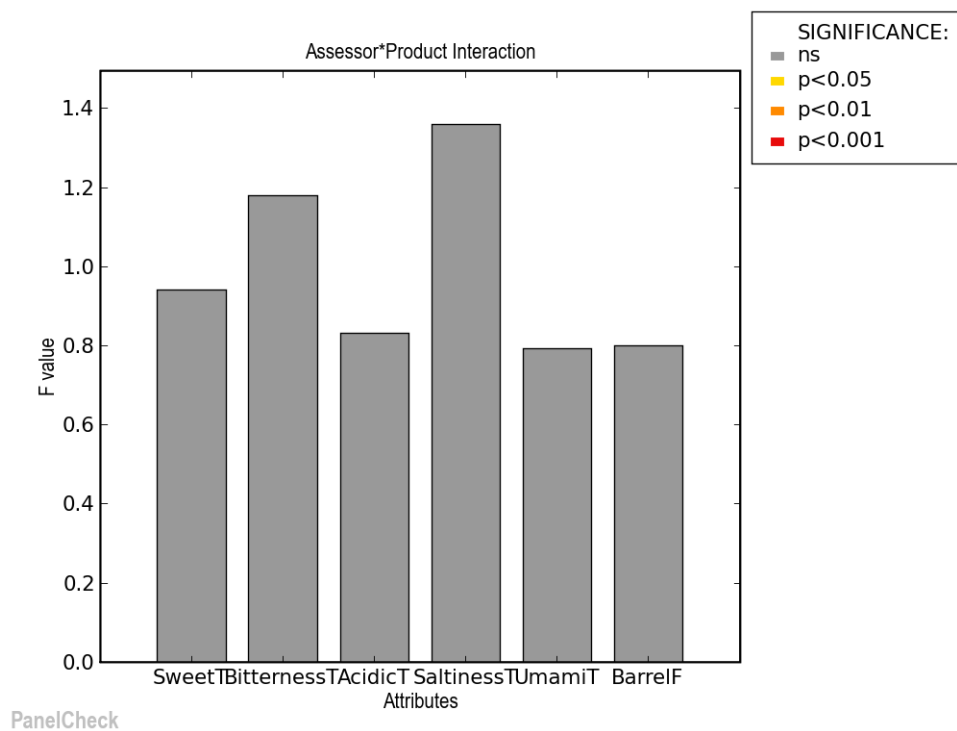


Figure 5: The PanelCheck result for the Assessor\*Product interaction when evaluating the attributes. Describes if the perception of the product differs dependant on the assessor.

The Tucker1 plot common scores gives an indication on how the products relate to each other according to the assessors' perception of them. Figure 5 shows that the samples are similar, since the scale the x- and y-axis are -4 – 6 and -6 – 3, respectively.

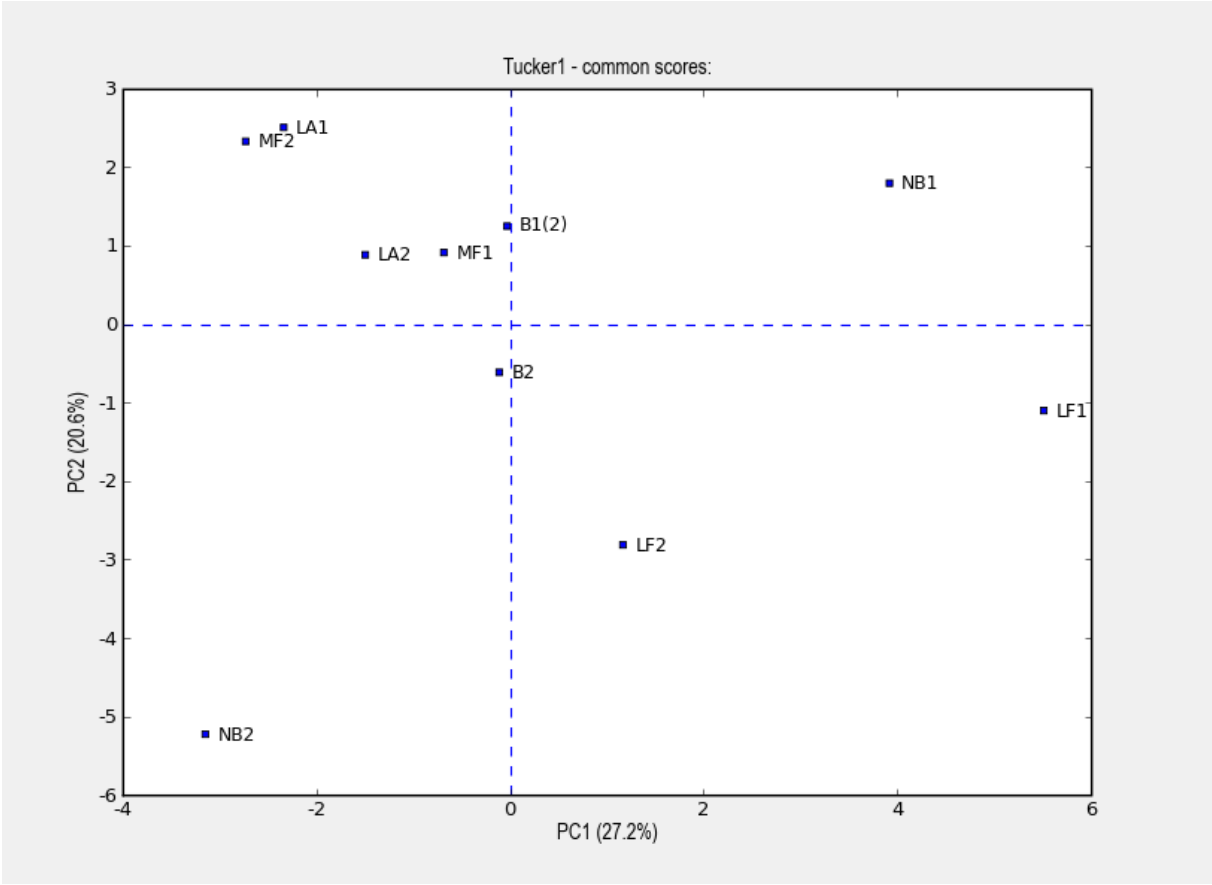


Figure 6: Tucker1 plot - common scores. Evaluates how similar the products are as perceived by the assessors.

The Tucker1 correlation plots shows how well the assessors agree regarding the different attributes, and how the assessors relate to each. The assessors agree when they are grouped tightly between the two blue circles that can be seen in figure 6. The assessors can be grouped in smaller group which means that there is some disagreement between them. It is apparent in figure 6 that there was not much if any agreement in the panel.

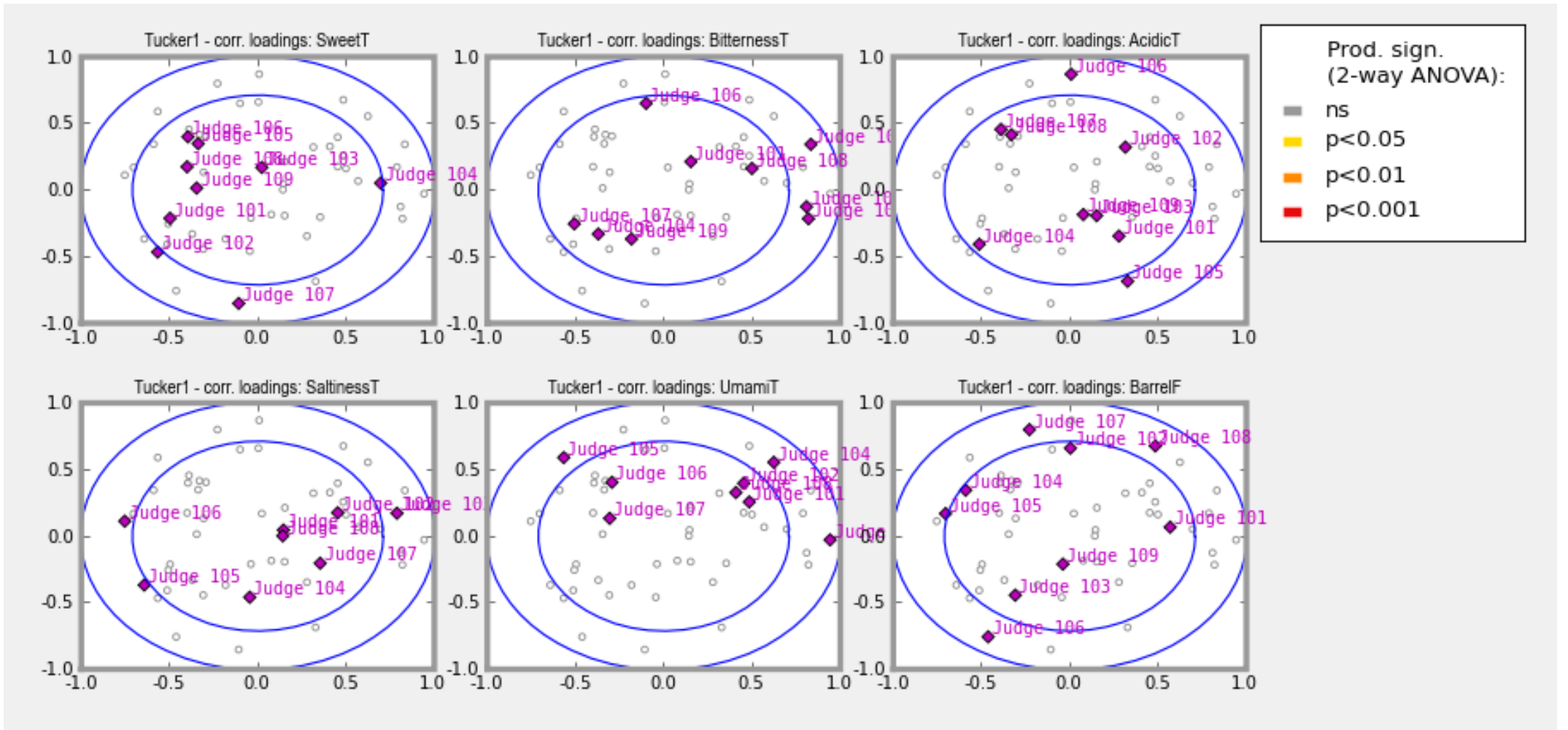


Figure 7: The Tucker1 correlation loading plots. These show how well the different assessors agree relative to each other regarding the chosen attributes.

The  $p^*$ MSE plot evaluates the method itself regarding the discrimination and the repeatability of the sensory analysis when examining the attributes. The plot is evaluated by dividing it into four quadrants (I, II, III, IV) as described in section 1.4. The plots in figure 7 indicate that there was mainly poor discrimination between the samples, while the repeatability was slightly sub-par for most of the attributes. The plots for acidic taste and barrel flavour show that the assessors have especially poor discrimination and poor repeatability for these attributes.

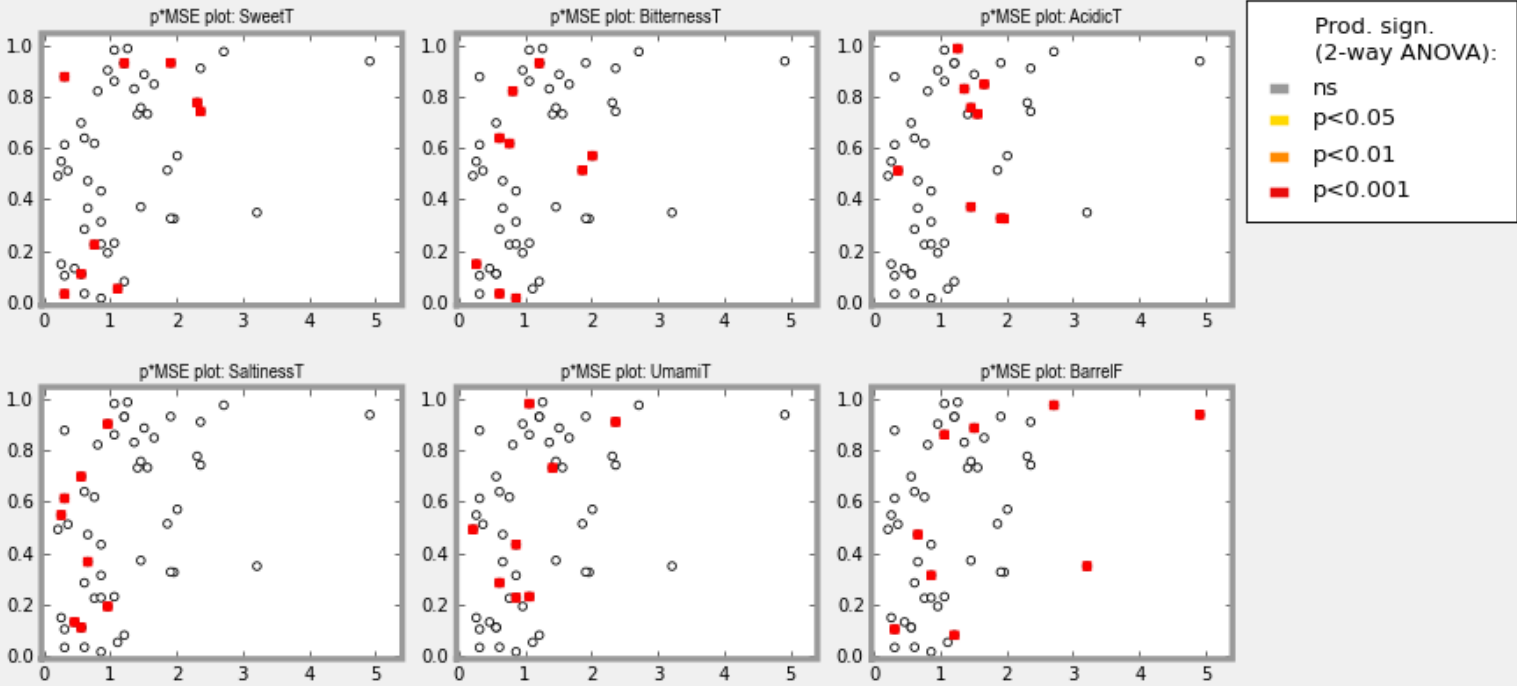


Figure 8:  $p^*$ MSE plots. The red squares are the compiled assessor data, while the black circles are all the datapoints for that given attribute.



### 3.2.2 CATA-test results

The CATA-test results were compiled in excel and displayed in a bar-chart. These can be viewed in their entirety in A-3 – A-6. When it came to aroma, the CATA results show that the assessors detected mostly malt, citrus, hops and tropical fruits. Vanilla was also detected to a lesser extent along with herbal and smoke aromas. Figure 8 shows that the samples were alike a certain extent, with some outliers that differentiated themselves from the rest, namely MF2 (first series) and B2 (first series).

Regarding flavours, the CATA-test shows that citrus, malt, tropical fruits, hops and alcohol were most abundant. Young/fresh oak, vanilla and orange flavours were predominant in some of the samples, varying by the type of wood used during the maturation. Figure 9 illustrates this. The flavour profiles of the different samples are also much more similar here than in the aroma profiles in figure 8.

The colour of the samples was also evaluated through CATA (A-5), with most of the samples either being characterized as “golden yellow”, “light brown”, or “golden brown”, with “golden yellow” being most favoured.

The texture and complexity of the samples were the last category to be evaluated (A-6). Here, the assessors deemed the texture of the beer to be average when comparing them to normal beers. Some of the samples were deemed by some to be complex and nuanced, while others had thinner texture with a simple or little complexity.

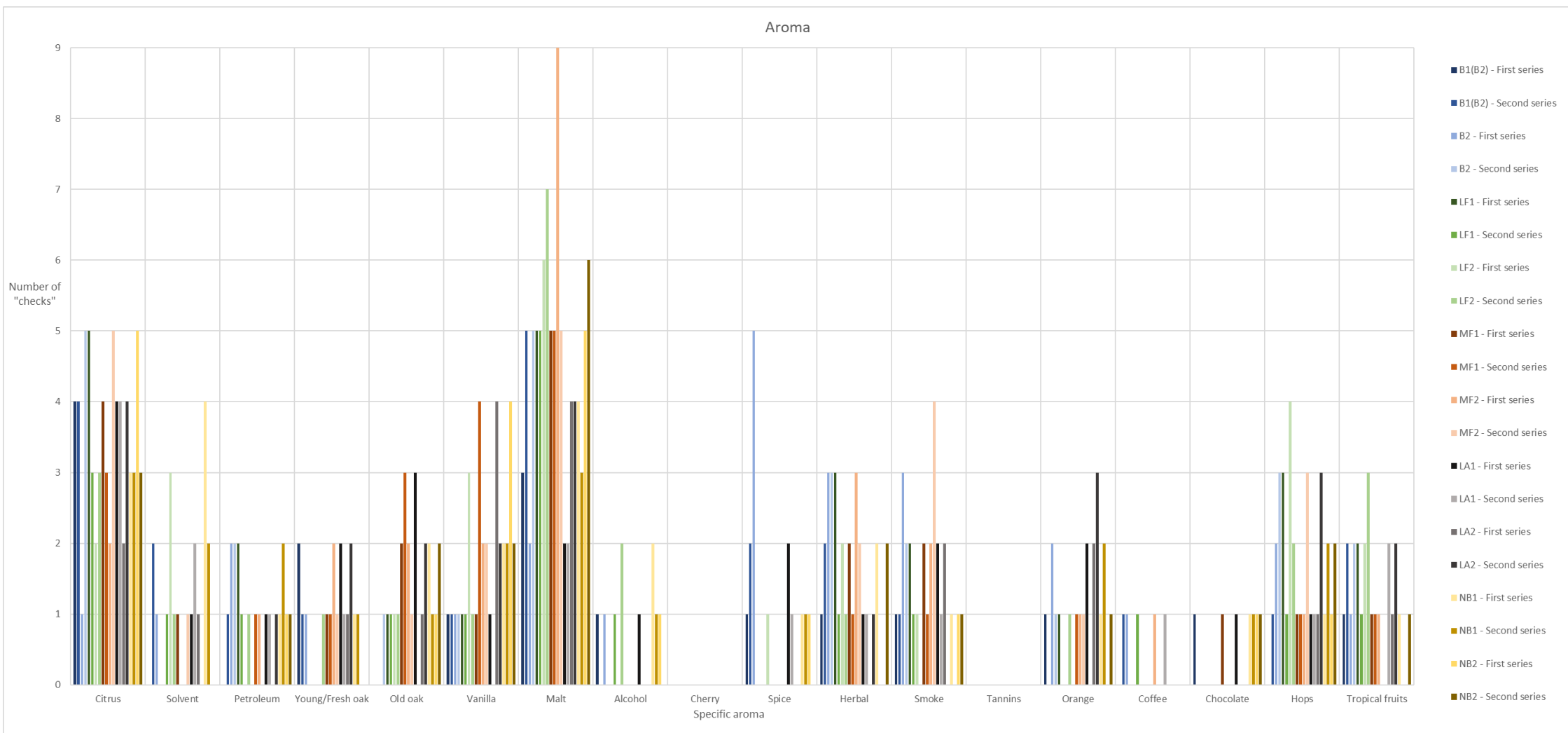


Figure 9: Compiled results of the CATA-test concerning the aromas present in the samples. Blank samples are coloured blue, Light French are coloured green, Medium French are coloured orange, Light American are coloured grey, and Norwegian birch are coloured brown.

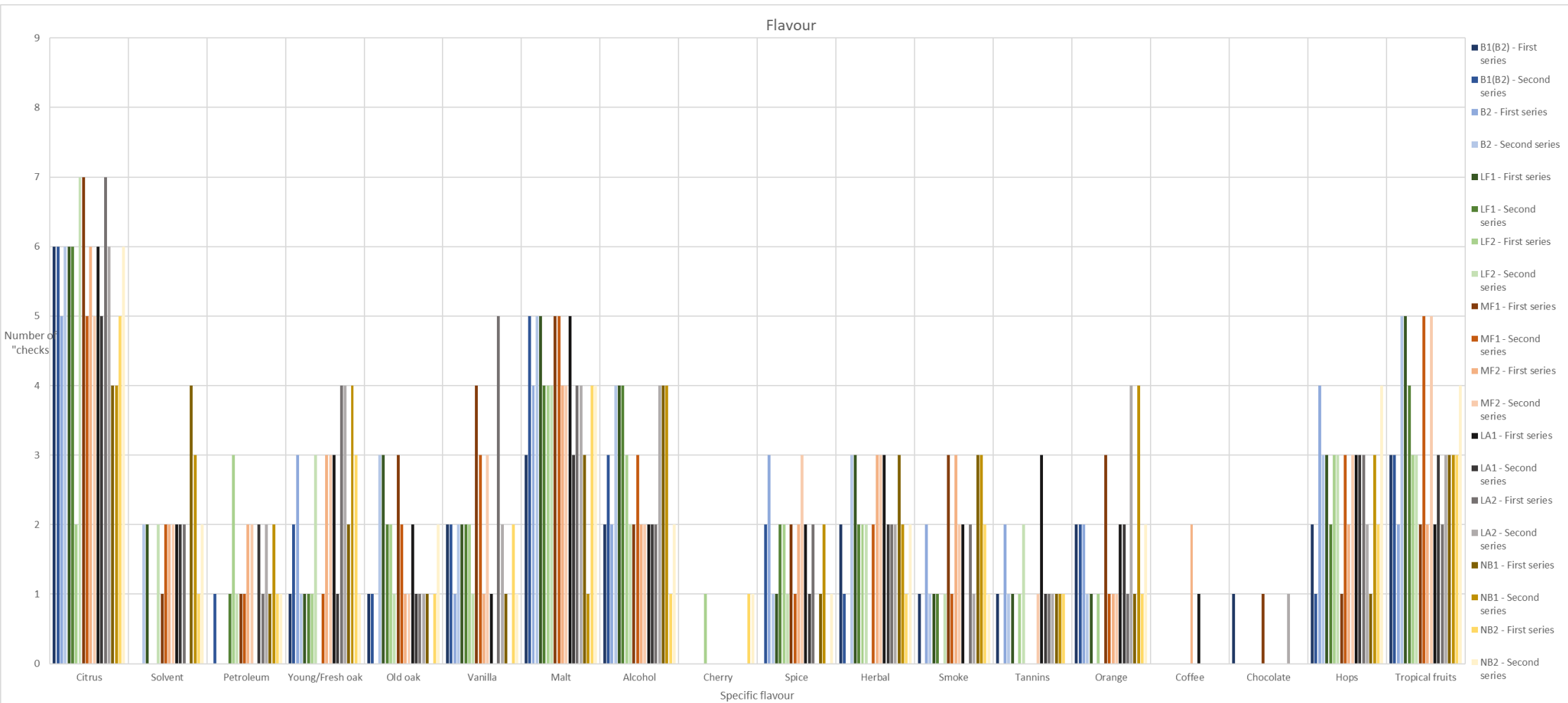


Figure 10: Compiled results of the CATA-test concerning the flavours present in the samples. Blank samples are coloured blue, Light French are coloured green, Medium French are coloured orange, Light American are coloured grey, and Norwegian birch are coloured brown.

### 3.3 Chromatography

The results can be found in their entirety in Appendix B (B-1).

#### 3.3.1 HSGC-FID

The chromatography quantified a series of organic compounds, where several of them can have a noticeable impact on the aromas and flavours of the samples.

*Table 5: Compiled results from the HSGC-FID analysis. It shows the different compounds that were quantified, the detection threshold for those compounds in beer, how many were over that threshold, and which sensory properties they can impart to the beer.*

Compound	Lowest detected value (ppm)	Highest detected value (ppm)	Average value (ppm)	Detection threshold (ppm)	Number of samples above detection threshold	Taste/Aroma/Flavour Profile	Comment
Acetaldehyde	3,332	252,923	39,439	5 - 20	28	bruised apples, green apples, grassy, cidery, raw apple skin and more	Either from yeast or infection
Acetone	0,020	0,541	0,115	-	-	nail polish, solvent, "warming"	
Dimethylsulfide	0,002	0,017	0,008	0,025 - 0,050	0	cooked and processed vegetables like cooked broccoli, tomato sauce, canned vegetables and more	
2-methyl-propanal	0,004	0,110	0,038	15	0	cereal-like, straw-like	degradation of hops

Compound	Lowest detected value (ppm)	Highest detected value (ppm)	Average value (ppm)	Detection threshold (ppm)	Number of samples above detection threshold	Taste/Aroma/Flavour Profile	Comment
1-propanol	41,863	49,496	46,578	-	-		Common by-product from fermentation
Ethylacetate	20,905	45,085	31,162	8 - 42	42	bruised apples, green apples, grassy, cidery, raw apple skin and more	yeast growth, wild yeast, low O <sub>2</sub> %
2-methyl-propanol	70,850	83,388	79,074	80 - 100	18	Alcoholic, fusel	yeast
3-methyl-butanol	0,009	0,039	0,020	0,003 - 0,015	42	malty	reaction between leucine and reductones
2-methyl-butanol	0,016	0,128	0,097	0,01-0,1	42	almonds, cocoa, coffee, nutty, musty	
3-methyl-1-butanol	145,433	180,446	159,412	70	42	fruity, banana	
2-methyl-1-butanol	40,632	47,249	44,607	65	0	banana, solvent, medicinal	
Isobutyl	0,031	0,067	0,046	1,6	0	sweet, fruity, banana	
2-hexanol	0,506	1,049	0,745	400	0	coconut	Typically, from wood-aging beer
Butyl acetate	0,008	0,366	0,131	3,5	0	solvent, banana, acetone, sweet	

Compound	Lowest detected value (ppm)	Highest detected value (ppm)	Average value (ppm)	Detection threshold (ppm)	Number of samples above detection threshold	Taste/Aroma/Flavour Profile	Comment
1-hexanol	0,043	0,398	0,109	400	0	coconut, green leaves	unpleasant
Isoamyl acetate	0,495	1,322	0,823	1,2 - 1,6	1	banana, apple, solvent, esters	Varies for type of beer
Ethyl hexanoate	0,044	0,106	0,071	0,21 - 0,23	0	apples, aniseed, sweet, fruity, esters	Varies for type of beer
Ethyl heptanoate	0,001	0,002	0,001	0,4	0	fruity, fatty, perfumed	
Ethyl octanoate	0,056	0,097	0,079	0,9	0	apple, sweet, fruity	

Among the 19 compounds detected by the HSGC-FID, only 7 compounds were over the detection threshold in beer in 1 or more samples. None of these compounds have sensory properties that overlap with the typical sensory properties from phenolic sources.

### 3.4 Folin's method

The results for Folin's method can be found in their entirety in Appendix C.

Folin's method quantified the phenolic content in the samples to be between 1031,16 mg GAE/100mL (LA121) and 1290,807 mg GAE/100mL (LF111), with most of the samples being between 1140mg GAE/100mL and 1190 mg GAE/100mL (25 of 42 samples), across all replicates over the 6-month period. The phenolic content stayed relatively consistent over the 6-month maturation period, with all the parallels varying at most by  $\pm 6,5\%$  within the series. All the 6-month samples also stayed within  $\pm 3,6\%$  of the starting samples (B00X), showing no significant increase or decrease in phenolic content (table 5).

Table 6: The development of the phenolic content over time. The starting concentration is the average of the B00X samples.

<b>Sample</b>	<b>Average starting concentration</b>	<b>Average final concentration</b>	<b>Difference</b>	<b>Percentage difference</b>	<b>Standard deviation over the maturation</b>	<b>Standard deviation percentage</b>
<b>B161</b>	1151,86	1167,30	15,44	1,32 %	18,94628544	1,62 %
<b>B261</b>	1151,86	1167,30	15,44	1,32 %	29,39002329	2,52 %
<b>LF161</b>	1151,86	1144,14	-7,72	-0,67 %	72,48905416	6,34 %
<b>LF261</b>	1151,86	1117,47	-34,39	-3,08 %	31,45133409	2,81 %
<b>MF161</b>	1151,86	1156,77	4,91	0,42 %	34,51402339	2,98 %
<b>MF261</b>	1151,86	1166,60	14,74	1,26 %	18,95494759	1,62 %
<b>LA161</b>	1151,86	1178,53	26,67	2,26 %	73,9344123	6,27 %
<b>LA261</b>	1151,86	1192,56	40,70	3,41 %	22,28737755	1,87 %
<b>NB161</b>	1151,86	1118,88	-32,98	-2,95 %	25,26640655	2,26 %
<b>NB261</b>	1151,86	1124,49	-27,37	-2,43 %	13,22051376	1,18 %

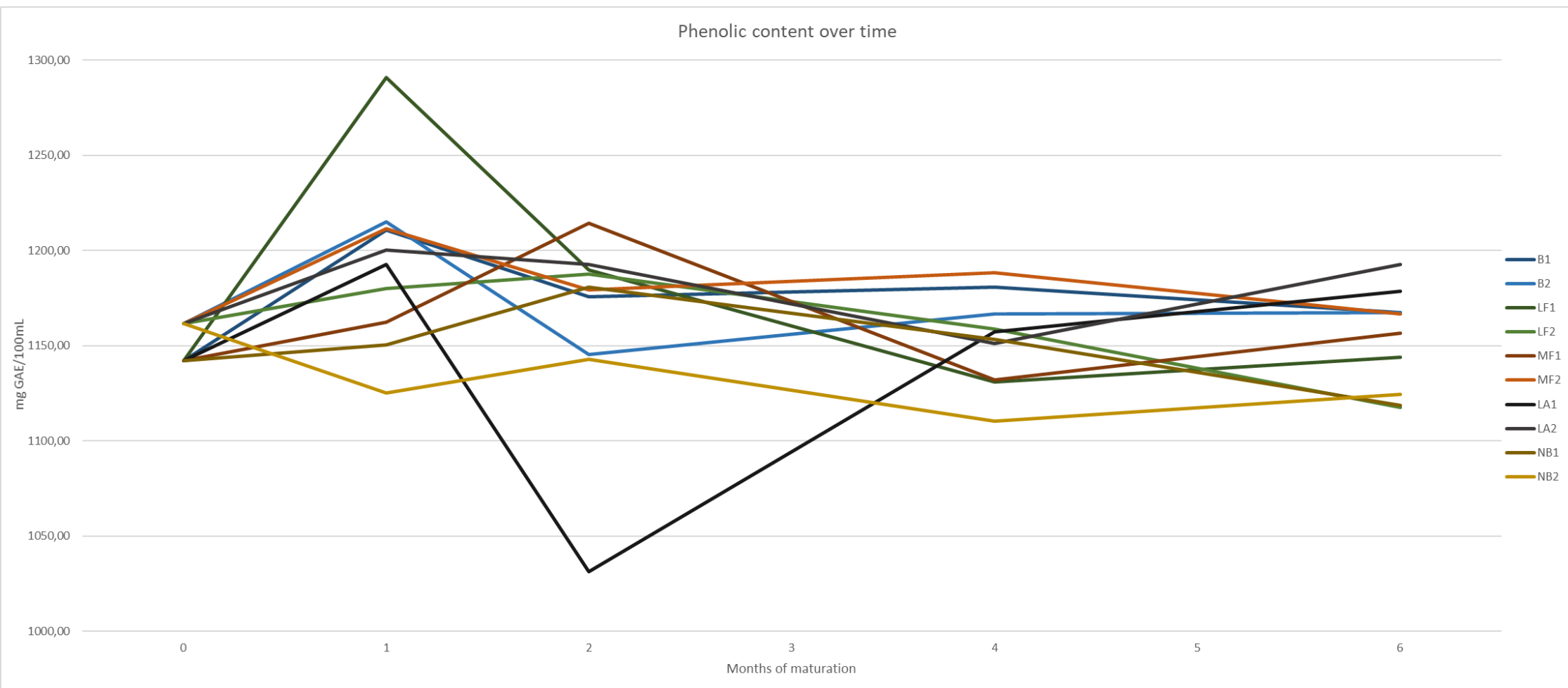


Figure 11: Phenolic content over time. Visually presents the changes in phenolic content for all the types and parallels over the course of the maturation period.



## 4. Discussion

### 4.1 The brewing process

As stated in section 2.1.2., there were a major issue during the mashing with determining the SG of the wort. Using “BeerSmith 2” to calculate the quantities, the SG should be in the area of 1,080 - 1,100 after the mashing process. During the first session, the measured SG was measured to be 1,084. The SG is however dependent on the efficiency of the brewing, with the average brewing efficiency is in the range of 75% - 85%. The readings in the 1,050 – 1,070 range would indicate an efficiency of about 60% or lower. Adjusting the efficiency in “BeerSmith 2” to match the measured SG from the first session, the SG matched at 74%. This is not terrible but could be improved. This makes equipment malfunction or misuse a far more likely cause of the fluctuations.

The refractometers use absorbance to calculate °Brix, and the absorbance of a liquid can vary with temperature. If the efficiency is set at 80%, which would be reasonable considering the equipment at the pilot brewery at NMBU, then it is possible to calculate an approximation of what the SG could have been. The recipe described in section 2.1.1., tables 1 and 2, would as stated have a calculated SG of 1,091. There was added about 2 kg of malt extract and 1 kg of refined sugar during the second session. If these are added back into “BeerSmith 2” and converted back to weight per 20L instead of weight per 50L, the calculated SG would be 1,105. Put in perspective, this equals an ethanol increase from 10,4% ABV to 12,5% ABV. This would explain the Anton Paar readings where the tested samples had ABVs from 10,85% ABV to 11,07% ABV. Accounting for the lower SG from the first session, there stands to believe that SG after the second session was above a value of 1,100.

The reason for assuming that the efficiency is the same in both sessions is again based on the equipment, since there are not many opportunities to make practical mistakes. There was however a lack of personal experience regarding this specific setup. The supervisor for this project was also present during both sessions, apart for the breaks during the boiling itself. Therefore, a difference of efficiency of 14% is very unlikely, and the malfunction of equipment is far more likely.

However, despite the technical difficulties, the result of having beers in the range of 10,85% ABV – 11,07% ABV is very much acceptable considering the assumption in section 1.1.1. concerning the concentration of ethanol needed to extract the phenols.

## 4.2 The aging process

To ensure that the wooden chips were sterilized, the chips were boiled for 1 minute while in the dry-hopping bags. This did change the colour of the water quite drastically. This does indicate that some percentage of the phenols was drawn out of the wood, leaving it with less to infuse with the beer. While there is evidence in section 3.2 to indicate that there are some phenols infused in the beer, there could possibly be more if the wood was not sterilized.

Due to COVID-19, all the samples for both the GC-FID and Folin's method were taken out by the supervisor for this project, since all students were strongly advised to stay home during most of the 6 month period from March until September. The supervisor did say that the samples were not necessarily taken the same distance from the dry-hopping bag containing the wooden chips, though a slight rotation of the beer was done before sampling. This could explain some of the fluctuations in the results in section 3.4. There is however another unforeseen circumstance that renders this point inconclusive. This will be further discussed in section 4.5.

## 4.3 The sensory analysis

After taking the courses related to sensory science at NMBU, it was apparent that this was an important part of this project. Since beer is classified as a food item, it is only proper that it should be evaluated through taste. The evaluation of food items can be divided into two areas, sensory analysis with a trained panel or consumer tests/liking, dependant on if the test is analytic or hedonic as described in 1.4. Due to cost restrictions, the first option was off the table before the project even started. This option will however be relevant in section 4.6. The only option then was to use consumers, or in other words an "un-trained" panel.

But there was an idea that bloomed at this obstacle. Using the established contacts in the Vinmonopolet could prove useful, since the people there have varying degrees of experience regarding tasting. This means that people recruited from this group can be considered "semi-trained", since they have considerable experience from tasting many kinds of products, even products with small noticeable differences. A panel of this sort do have pros and cons though.

First pro, it is cheap and relatively easy to schedule with this kind of panel. Secondly, they can distinguish between similar products to a certain extent. In a study from 2008, a research group investigated the effects of using a trained vs an untrained panel. They found there was no significant difference between the assessor group when it came to "matching performance" (Lelièvre, et al., 2008). This could indicate that when the differences are very small, the

advantage of using a trained panel over an untrained one diminishes. Since the differences between the products were predicted to be quite small, there would not be a huge loss to use a panel that can be classified as “semi-trained”. Thirdly, it would probably be a shock to the untrained panelists to experience the full setup with the booths in the specialized room mentioned in section 2.2.1. This could negatively affect the assessment by discouraging the assessors. This also moves into the fourth point, which is the potential discouragement when the panelists realize how similar the samples were. Finally, even a “semi-trained” panel will probably struggle to rinse the pallet enough to give an even assessment throughout the entire tasting session. This session was scheduled to take about 2,5 hours, including the standardizing. This is a long time to taste 20 products that were based on the same recipe while assessing each sample equally.

These suspicions were confirmed in the comments made by the assessors. The consensus was that it was a lot more difficult to separate the products than they had imagined. Most of them agreed that the citrusy flavours and aromas were overpowering the other flavours and caused an unexpected level of fatigue.

The serving glasses also proved to be a challenge for the assessors. These glasses were 4 cl shot glasses which are standard for these kinds of tests. This made it very difficult to smell the samples properly, and therefore give an incomplete or inaccurate assessment of the samples.

The results that the assessors produced during the CATA test are rather interesting, however. As mentioned earlier, there was a lot of difficulty to determine the aromas of samples. There were still predominantly aromas of malt, citrus and tropical fruits. These can be associated with the finding of the HSGC-FID, where there were significant concentrations of esters, aldehydes and higher alcohols that can explain these aromas (discussed further in section 4.4.1). Other like vanilla, smoke, petroleum, young/fresh oak and old oak makes some appearances, but since there is not a significant number of observations they cannot be weighted. The flavours did however not have this problem as aromas regarding choice of glassware. The assessors perceived an abundance of citrus-, malt-, hops- and tropical fruit flavours. There is also a significant number of detections of vanilla, young/fresh oak, solvents, alcohol, orange, spice, herbal, old oak and smoke. Alcohol, orange, herbal and solvent (to a certain degree) flavours can be attributed to the findings by the HSGC-FID. Vanilla, young/fresh oak, spice and smoke however cannot. The perception of these attributes

does indicate that there are some phenolic compounds present in the beer that influence flavour, even though there are not enough “checks” to give a conclusive answer.

The PanelCheck analyses, as mentioned in section 1.4, the data from the 9-point scale portion of the survey and then evaluates the products, the assessors and the method.

The first one was the 2-way ANOVA. The assessor effect had significant differences across all attributes, meaning that the panelists all used the scale differently for the attributes. This is not such a detriment as it might seem since people perceive tastes and flavours differently. For instance, the perception of how bitter coffee is can differ from one person to another. The product effect does indicate that there were no differences between the products regarding tastes and “barrel flavour”. This is further emphasized with the spider-plot in Appendix A (A-7). The assessor\*product interaction shows that there were not any significant differences between the assessors when they evaluated the products, even for the “barrel flavour”. The attribute “barrel flavour” seems to be contradicting itself. The CATA analysis does indicate that only some of the assessors perceived a “barrel flavour”, be it young/fresh oak or old oak. This might be due to the inexperience of the panelists with this type of testing.

The Tucker1 plots were evaluated next. The common scores might indicate that there are some differences between the products. LA1, LA2, MF1, MF2 and B1(2) are for instance all clustered up in the top-left quadrant. The problem here is that PanelCheck automatically scaled the plot down in order to better visualize the differences, as seen by the -4 – 6- and -6 – 3 scales on the x- and y-axis, respectively. This plot is normally scaled from -20 to 20 on both axes. This does support the 2-way ANOVA product effect that there is no significant difference between the products. The correlation loadings show that the assessors do not really agree on any attribute. This further indicates that they lack experience and/or training.

Lastly is the p\*MSE plots. The assessors had good repeatability when it came to saltiness, but poor discrimination. This is expected since saltiness is not a typical attribute found in beer. The tendency of having difficulty discriminating continues bitterness, umami and sweetness, gradually getting worse at replicating the results. For acidic taste and barrel flavour, they have both poor repeatability and poor discrimination. This is again due to their inexperience for this type of testing. The method was probably not ideal considering the nature of the assessors. Due to time-restrictions, the standardizing process before the tasting was not as thorough as could have been. The panelists should ideally have been involved in the

development of the vocabulary as well, but this was sadly cut from the preparation due to unavailability and time-restrictions due to work hours.

The descriptive 9-point scale results can therefore be called inadmissible, since they both contradict the results of the CATA test, and they performed poorly regarding repeatability and especially for discrimination.

The statistical tendencies are still there though, indicating that there has been some extraction of phenols. Vanilla, smoky flavour, and astringent flavour (tannins) originate most likely from phenolic extraction, meaning there probably is vanillin, eugenol, guaiacols and/or other volatile monophenols in the beer. The assessors have proved as mentioned earlier not to be able to give a conclusive result, but this can mean that they did not know what they were looking for in the first place. Either way, the number of sensory attributes perceived that can be linked to phenolic compound does warrant further research (discussed further in section 4.6).

## 4.4 Chromatography

### 4.4.1 HSGC-FID

The HSGC-FID gave some interesting results, even though they may not seem relevant to the phenolic content in the samples. The reason why they are interesting and even necessary is that flavouring components such as phenols, aldehydes and organic acids tend to overlap with each other regarding certain sensory properties or -attributes. Therefore, it is important to cross-reference these results with the results of the sensory analysis to check if some attributes previously thought to come from phenolic compounds might be from other volatile compounds.

19 substances were quantified where most were aldehydes, esters or higher alcohols. Of these 19 compounds, only 7 were on average over or around the perception threshold for their corresponding attribute, these being acetaldehyde, ethylacetate, 2-methyl-1-propanol, 3-methyl-butanol, 2-methyl-butanol, 3-methyl-1-butanol and isoamyl acetate. These 7 can result in flavours like apples, grass, fruits, bananas, malty, fusel and solvents. Acetone does not have a documented perception threshold in beer, so this could also contribute flavours of solvent and/or nail polish remover. Regardless, none of the 8 detectable, nor the 19 substances in total, overlapped with the previously established typical sensory attributes due to phenolic compounds.

There were detected high levels of acetaldehyde in samples NB141, LF161, LF261, MF161, MF261, LA161, LA261, NB161, all being  $\geq 99$  ppm. Acetaldehyde is common in all types of beer to varying degrees, but they tend to vary between 2 ppm and 15 ppm. 17 of the 42 samples analysed by HSGC-FID were over 30 ppm, and the majority of those are 4-month- or 6-month samples. The first plausible explanation for this is that the ethanol could be oxidized back into acetaldehyde. Since the lids were opened over the course of the 6-month period, fresh oxygen could have entered the maturation tanks and started the oxidation. This does not explain the  $\geq 99$  ppm however, but a microbial contamination might. An infection of either bacteria or wild yeast could have led to such high levels. This makes especially sense with sample NB161 which was quantified at 252,923 ppm, since the birch used was only boiled for 1 minute and otherwise untreated. NB261 was quantified at 50,122 ppm while using the same treatment. The possible reason for this disparity between NB161 and NB261 is that the wood that was cut from the log may not have been mixed sufficiently, i.e. NB161 received a larger percentage of contaminated wood compared to NB261.

#### 4.5 Folin's method

During the execution of Folin's method to determine the total phenolic content, there were some irregularities when compared to earlier uses of this method at NMBU.

As can be seen in appendix C-2, both the 150 mg/L and the 200 mg/L gallic acid standards are above an  $Abs_{765}$  of 1,0. There were two concentrations that were supposed to be in the standard curve, these being 250 mg/L and 500 mg/L, which had an  $Abs_{765}$  of 1,8 or above. Due to not knowing if these were within the linear area of the standard curve, these 2 concentrations were discarded. This was further tested against a sample from the MF series, the series suspected to have the highest phenolic content of all the samples. This sample was measured to be in the 0,350-0,400 range, meaning that the part of the scale that was needed was well within the linear range. When comparing this to a thesis published earlier this year, in its appendix E-11, the 500 mg/L measurement had an  $Abs_{765}$  of approximately 0,485 (Aasen, 2020). This is a 2,5 times stronger concentration with less than half the absorbance,  $Abs_{765}$  of 0,485 (500mg/L) compared to  $Abs_{765}$  of 1,737 (200 mg/L).

Such a disparity between earlier uses of this method, and the use connected to this experiment, is too large to overlook. After analysing the data, there are some explanations that are logical for this method. The method is very simplistic in its design, requiring only 3 reagents and an incubation time to be ready for measurements. A likely explanation is that

there was a weigh-in error when making either the Folin-Ciocalteu reagent, the buffer or the stock solution. This is the most likely cause of the high values. However, it is still highly improbable since these reagents were remade while trying to make sense of high readings. This was to no avail since they yielded the same results as during previous measurements. Another explanation, while being less likely, is that the oxidising properties of the Folin-Ciocalteu reagent could be compromised in some way. An even less likely option is the calibration of spectrophotometer itself. It was set to the correct wavelength, that being 765 nm, and was zeroed using milli-Q water. Even though I personally cannot say how often that specific spectrophotometer was used, I would think that this exact instrument would be regularly calibrated due the labs apparent usage, or at least that such a large disparity would be quickly discovered. However, there was not any evidence to help indicate which fault, either singular, plural or unmentioned, was responsible for this gap between expected and actual results. Since new standards were made, with no effect on the apparent problems, it was agreed upon that the analysis should continue as planned.

Even though the procedure continued, there were still some outliers. This especially concerns figure LF in appendix C-5, and LA in appendix C-7. Here, samples LF111 and LA121 had a large jump and a huge dive, respectively. This can however be explained. The flasks used for the maturation process were old glass containers varying from 15L to 20L of volume, which were also quite fragile. This combined with the 9L – 10L of beer in them meant that they were quite cumbersome to handle (see figure 1). They were stirred gently before extracting the necessary samples, but this may not have been enough to homogenize the beer completely, meaning that concentrations of phenols could be higher or lower throughout the fragile glass container. The opening of the flask was not very wide either, making it difficult to use any large utensils to stir the beer. Since there was only one taking out the samples as mentioned in section 3.2, it would be risky and difficult to ensure a proper swirl, and therefore a proper mix of the beer. The one who took out the samples was however methodical when it came to the extraction, taking the samples roughly the same distance away from the dry-hopping bag. This should have ensured an even series of datapoints, but this can be extremely difficult over a 6-month period without mechanical aid. The sudden increase in phenolic content in sample LF111 could have been the result of large release of phenols from the wood during that first month. This is however less likely than the first suggestion, because this should also have happened in the other containers as well to a larger or lesser extent.

Even the numeric values are questionable at best, they are still probably just shifted one way or the other. I.e. the relations between the datapoints are still highly relevant, and still valid for discussing the trends that happened during the maturation process.

When comparing the different samples to each other in general terms, there is evidence to suggest that the phenolic content has not changed to a significant degree over the course of the 6 months with all ending within  $\pm 3,6\%$  of the starting value, with a variation of  $\pm 6,5$  at the most over the duration. This could suggest that as the phenols from the beer itself kept oxidizing, the phenols from the wood diffused into the beer, supplementing it to roughly the same level. The most interesting aspect of this is that the blanks, B1 and B2, do not seem to have a decline in phenolic content either. They did not have any wooden chips, so that would indicate that this would not be the case. This would seem to lead to the conclusion that added wooden chips does not change the phenolic content in beer. This contradicts the findings mentioned earlier where the phenolic content should decrease by between 16% and 23% over the course of 6 months (Wannenmacher, et al., 2018). The absence of a 16% - 23% decrease would indicate that there was a significant supplement of phenolic compounds from the wooden chips, but as stated, it does not explain the same absence in the blanks.

#### 4.6 Further research

This project had its share of interesting moments, but the research on this subject is far from complete. There were limitations in the methods that only made it possible to establish trends and tendencies. This is the subject of 5.6, with an emphasis on alternate methods that could have given clearer results if this had been available.

There has not been done a lot of research regarding the phenolic contents of barrel aged beer and how this in turn affects the taste of the beer. According to Web of Science per 15.11.20, there have been written 2 articles with the searchable keywords of: “beer”, “barrel aged” and “phenol”. Google Scholar had a little over 3000 results for the same search words per 15.11.20, however there were a lot of cross references with the research of wines. This can then be considered a relatively new field within both chemistry and food sciences.

##### 4.6.1 Brewing and maturation

The brewing recipe and process itself was good enough for both a proof of concept and further investigation, except for the equipment malfunction in described in sections 3.1.2 and 5.1. This part of the experiment must be redone either way, so the malfunction would probably be corrected during the next brewing session. What is interesting first however is the



choice of style. The style “Belgian strong ale” is not actually defined as a proper style of beer and is more a descriptor for beers with Belgian character and a strong ABV. The obvious choice for a proper style related to this faux-style would be the trappist, which was traditionally well-balanced beer with a typical ABV of 9% - 11%. The quadruple trappists, which are mashed 4 times, would be an ideal candidate for this. Other interesting styles would be stouts, barley wines, and gueuze. All these styles have been barrel-aged in the past and could be an interesting insight into the composition.

The maturation and aging itself could use more refinement though. Wooden chips were used for this project, but they have some pros and cons. Wooden chips are cheaper, easier to handle, require less space, and often single use and therefore require less maintenance. It is however uncertain how realistic this is when comparing this usage to that in the industry. Therefore, both options of wooden chips and full-on wooden barrels needs to be considered. Another aspect that needs to be addressed is time. There are no rules regarding duration when it comes barrel-aging of beer like there is with scotch, Irish whiskey or champagne. For beer it can vary from two months as seen in at least one study (Sterckx, et al., 2012), to three years in another (Sanna & Pretti, 2014). In the study by Coelho et al., there is a small but observable differences in volatile phenolic content when comparing 16 month aging (ab1) and 11 month aging (ab3) (Coelho, et al., 2019). Since the main discernible difference is the aging duration, then it stands to believe that the beers in this experiment could have developed further given 6 – 12 additional months.

#### 4.6.2 Sensory analysis

During the sensory analysis there were mostly comments on how difficult this was to differentiate between the samples when they were so similar. This became even more difficult the longer the tasting lasted. This is most definitely because the panel’s inexperience with this kind of tasting. Then how could this problem had been circumvented? The answer is to use a trained sensory panel over the course of several days instead of the method used here.

Preferably, a trained panel consisting of professional beer tasters and/or professional sensory scientists. A couple of examples for why this would be preferable. When the vocabulary for this project was developed, there were only 2 people present and neither of them acted as panelists for the analysis. This made sense for this project. A trained panel however could be more involved during the vocabulary development, which give a more specialized vocabulary for a specific test. The panel could also be a lot more accustomed to tasting samples with miniscule differences, making for better descriptions of said samples. It can also be argued

that the recognition of different tastes also could have been better with a trained panel. The problem with using such a panel is that these are often quite costly and normally only available in the industry itself.

This is somewhat a contradiction to the article by Lelièvre et al. where they state that there is no significant difference in using a trained- or untrained panel when the differences are miniscule. It could be argued however that better preparations involving the panel could lead to a more conclusive result. The ability to taste if the sample has mandarin or orange flavour might not be that relevant but the experience such a panel has is very much a factor.

#### 4.6.3 Chromatography

During the search for relevant articles and references for this thesis, it became apparent that the preferred method for analysing phenols in beer was GC-MS with highly specialized columns over using GC-FID, but there is no consensus on which method is objectively better. As briefly described in section 1.5, a mass spectrometer (MS) has some advantages and disadvantages when compared to a flame ionization detector (FID). The most significant one for this project was availability, where the one option was a GC-FID. The available GC-FID could have been adapted to work better, with the use of a specialized column. This could not be done during this run due to the budget and available analytical methods. It would be interesting to see if this could give some quantitative results for which specific phenols were in the beer, either through GC-FID or GC-MS.

## 5. Conclusion

Earlier research, while not numerous, does conclude that there are phenols in beer naturally, there are phenolic compounds in wood, and they can be extracted into the beer. Volatile monophenols and phenolic aldehydes like vanillin, eugenol and guaiacols has been quantified in wood-aged beer.

There were a lot of tendencies that imply that there likely are phenols present in the beer due to the wood-aging and that they have influenced the sensory properties to a certain extent. The sensory analysis, while maybe lacking, does support this with the perception of the vanilla, smoke, spice, young/fresh oak and old oak sensory attributes. The abundant presence of citrus and tropical fruit flavours may have overpowered the more subtle flavours that the phenols may have provided. The use of a “semi-trained” panel may have been sub-optimal, but should however not have mattered to such a degree as it would seem (Lelièvre, et al., 2008).

Speaking of the potentially overpowering flavours, the HSGC-FID did establish that there were a lot of other compounds that can give those sorts of sensory attributes.

The analysis using Folin's method indicate that there are significant quantities of phenolic compounds in these samples and that the normal degradation of 16% - 23% has not taken place, meaning that the wood as at some level supplied the beer with more phenols.

This experiment has not given any concrete evidence or answers regarding the original hypothesis, that being "Which phenols are extracted from the wood itself during wood-aging, how much of these are extracted, and how this effects the flavours, aromas, and the overall impression of the beer". If the phenolic content in the blanks had gone down by 16% - 23%, then there would have been conclusive evidence that phenols has been extracted for the wood. While yielding no readings about phenolic substances, the HSGC-FID did however show to a certain extent that there were no non-phenolic compounds that could have overlapped phenolic compounds regarding sensory attributes.

Based on the evidence that is present however, the following conclusions can be made. Firstly, there are an unknown quantity of phenols in the beer as a result of wood-aging. Secondly, there is a high probability of vanillin being present, and by association also eugenol and guaiacols. Thirdly, this extraction has not affected the taste of the beer, but it has affected the flavour and aroma to an extent. Finally, the overall impression beer was altered by the wood-aging since the assessors deemed the wood-aged beers to be more complex than the blanks.

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## Appendices

### Appendix A – Sensory analysis

#### A-1: 3-digit codes used for the sensory analysis

*Table 7: The 3-digit codes used for the sensory analysis. They were created using a random number generator.*

<b>Sample</b>	<b>Code – First series</b>	<b>Code – Second series</b>
B1	556	876
B2	126	771
LF1	103	827
LF2	893	797
MF1	799	433
MF2	244	753
LA1	184	450
LA2	614	380
NB1	954	836
NB2	294	721

#### A-2: Survey used for sensory analysis

See attachment 1 or

[https://docs.google.com/forms/d/e/1FAIpQLSfwXI39IMw9nn7HI4s607wK7bSeNeZx\\_YPq2aQA3NtsuInqfw/viewform?usp=sf\\_link](https://docs.google.com/forms/d/e/1FAIpQLSfwXI39IMw9nn7HI4s607wK7bSeNeZx_YPq2aQA3NtsuInqfw/viewform?usp=sf_link)



### A-3: Summarized CATA results (Aroma) – compiled data

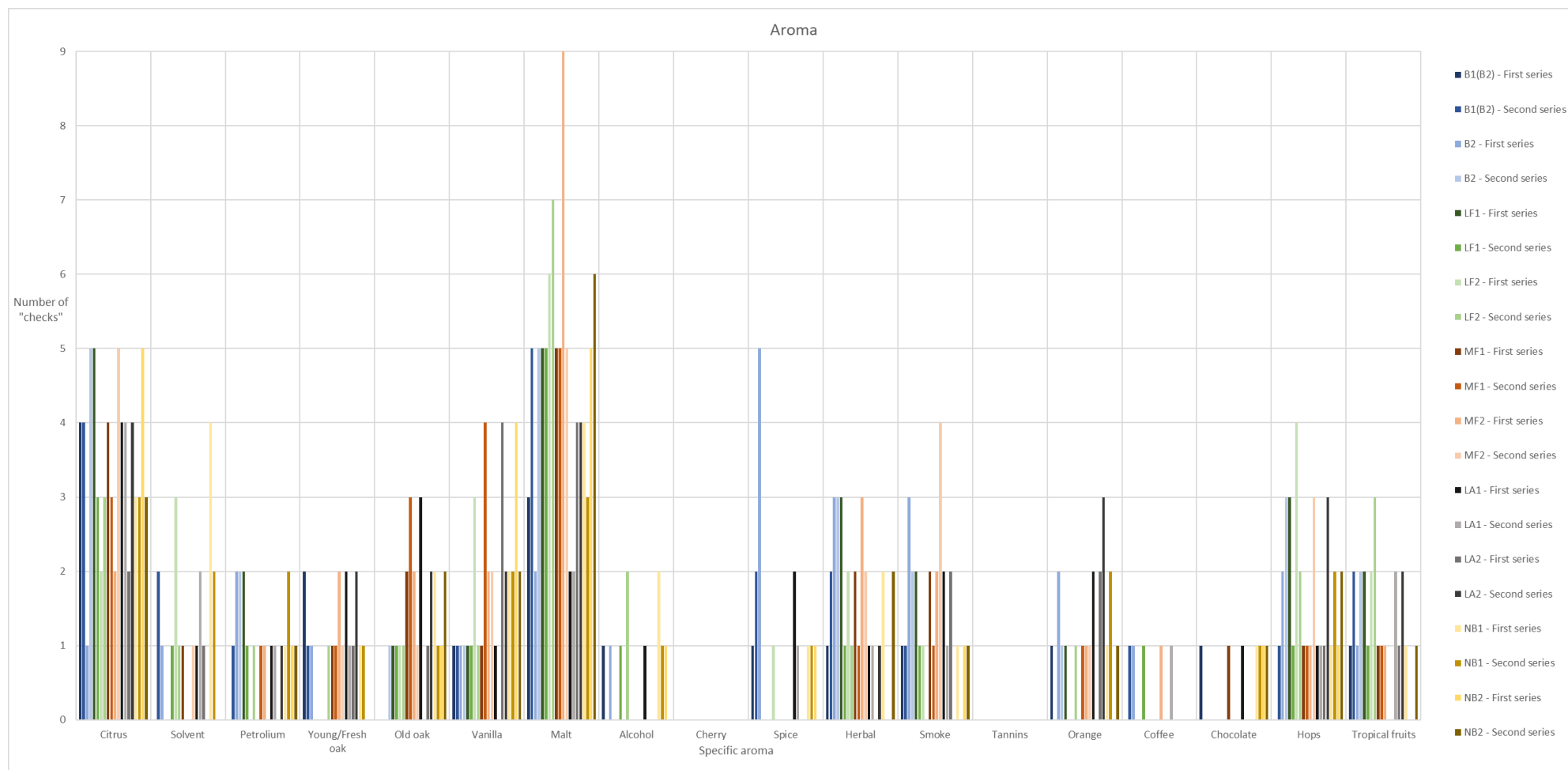


Figure 12: Compiled results from the CATA-test showing the perceived aromas.

A-4: Summarized CATA results (Flavour) – compiled data

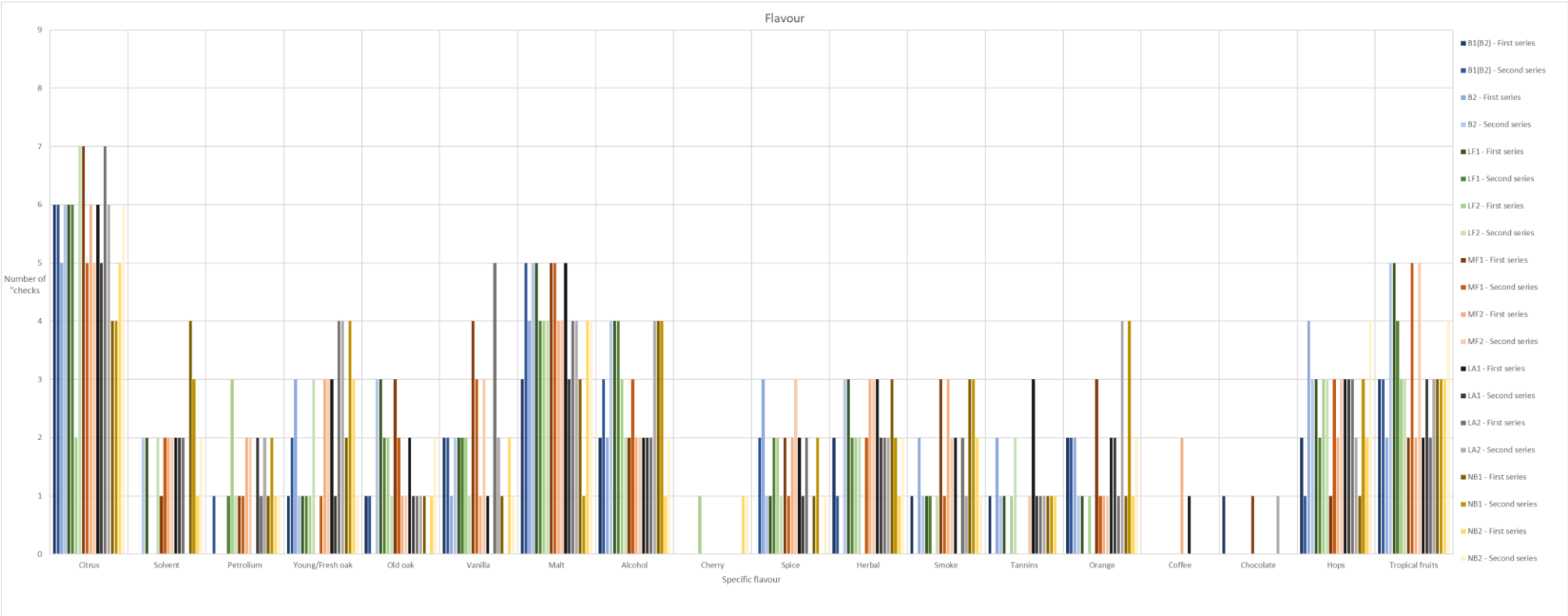


Figure 13: Compiled results from the CATA-test showing the perceived flavours.

A-5: Summarized CATA results (Colour) – compiled data

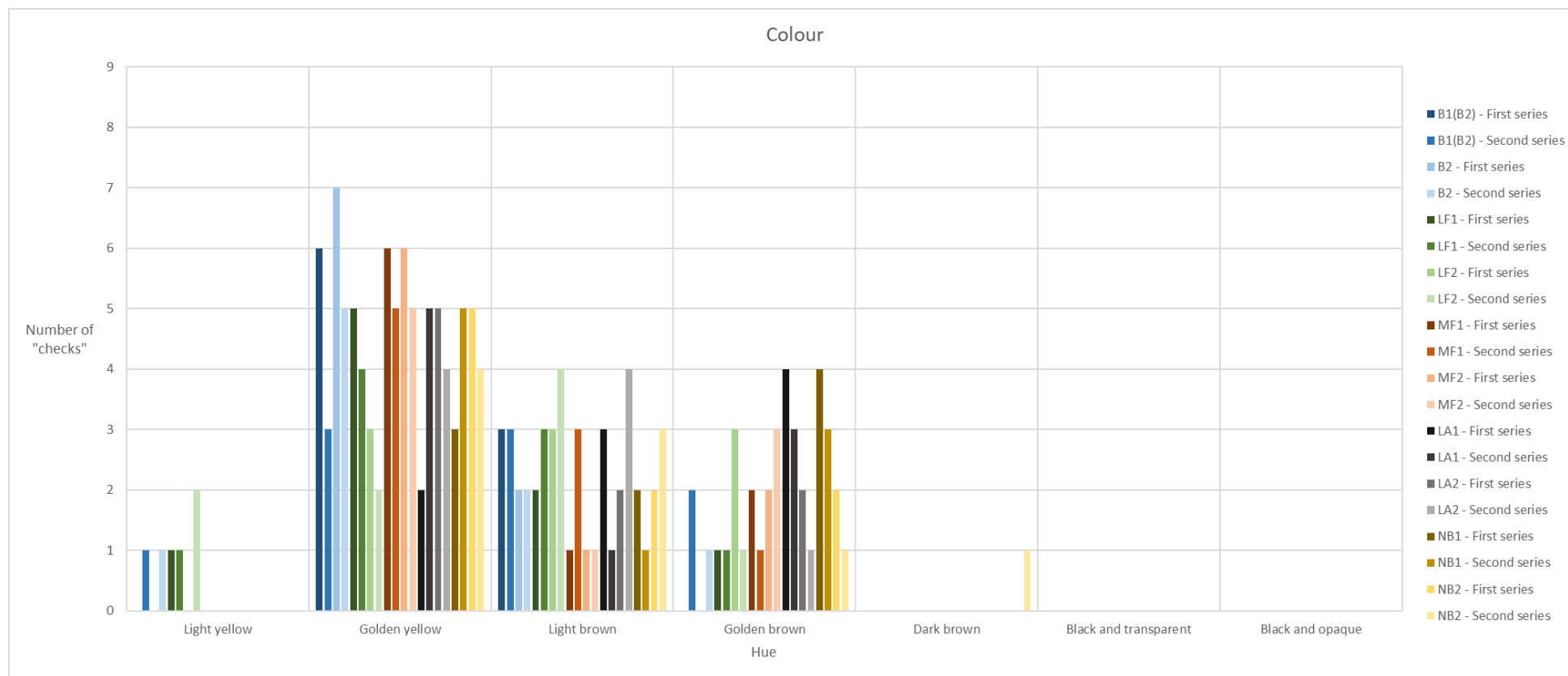


Figure 14: Compiled results from the CATA-test showing the perceived colour of the samples.

A-6: Summarized CATA results (Texture and complexity) – compiled data

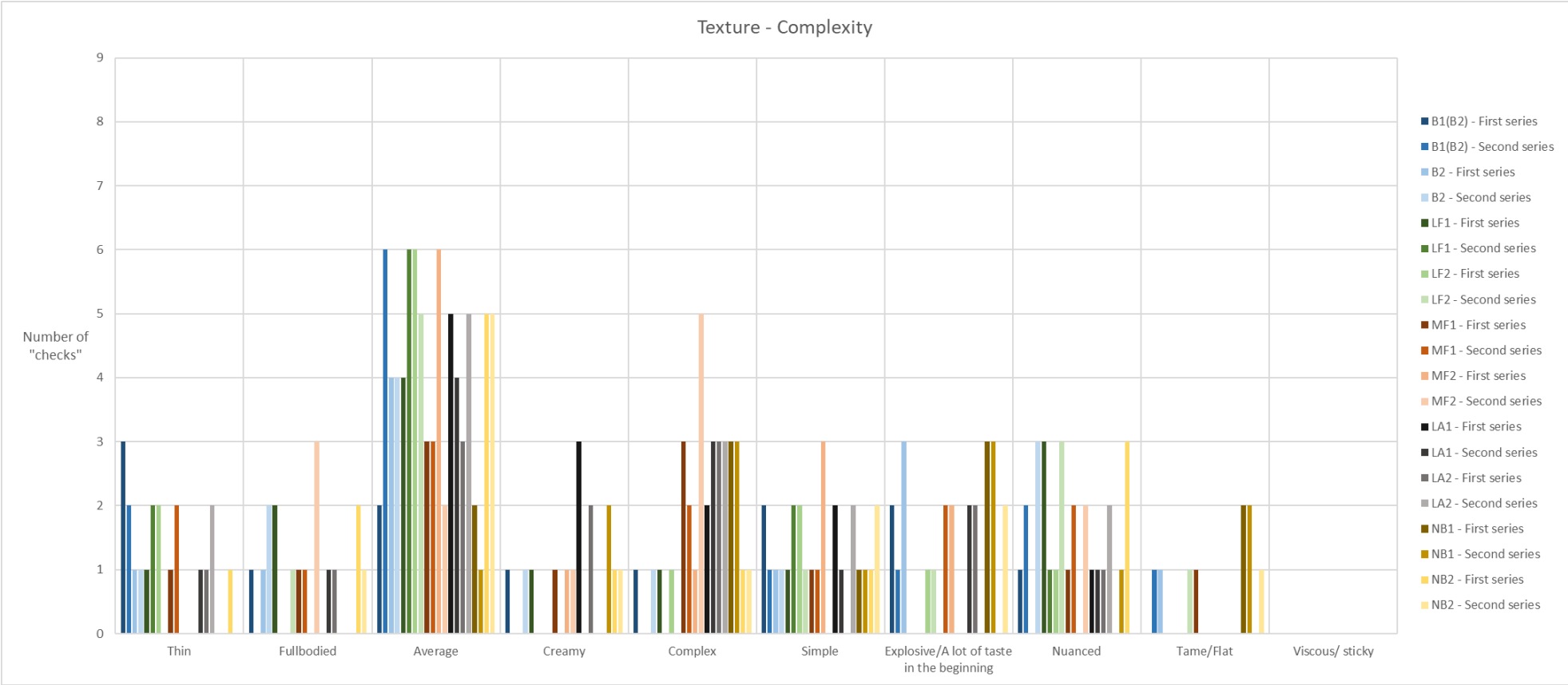
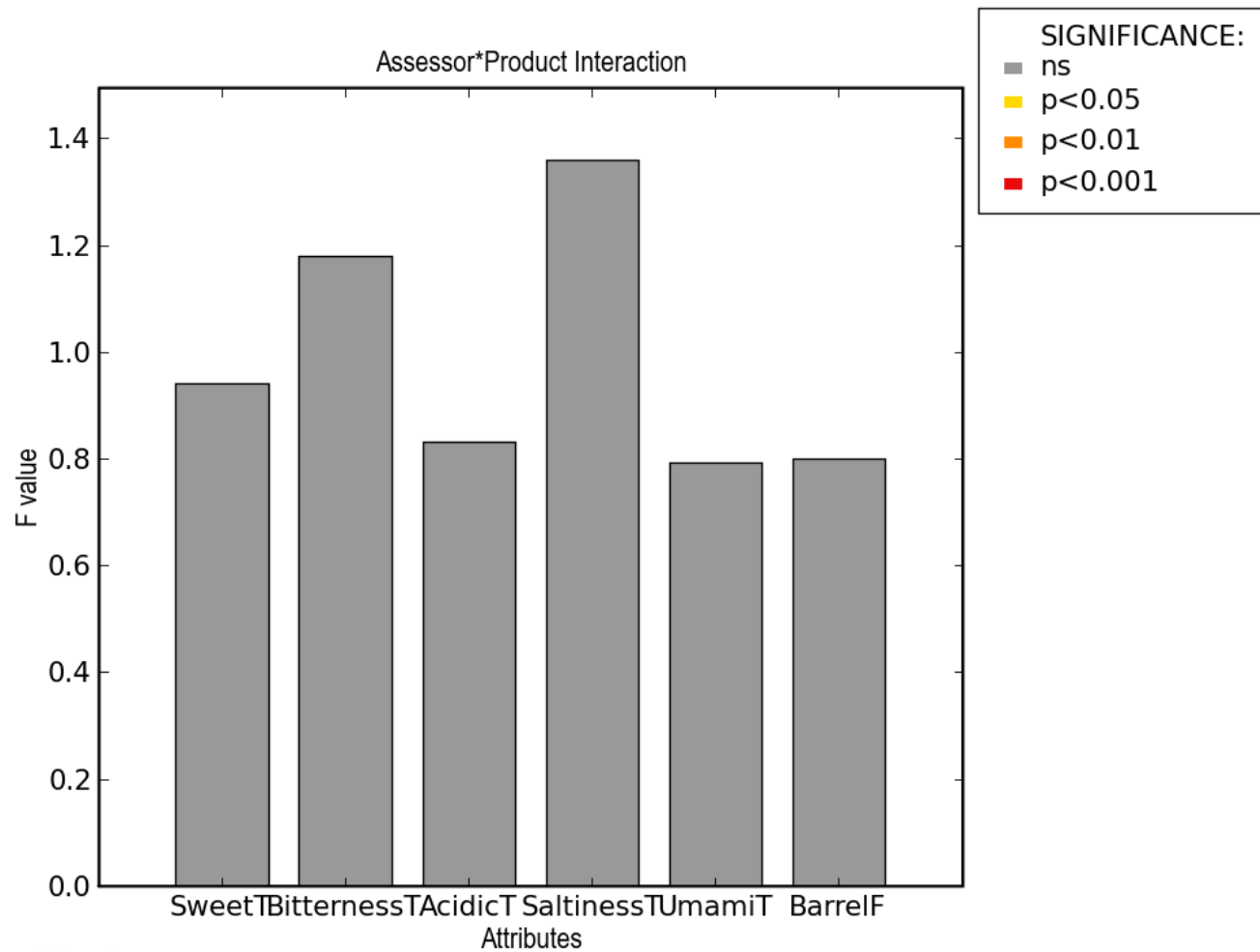


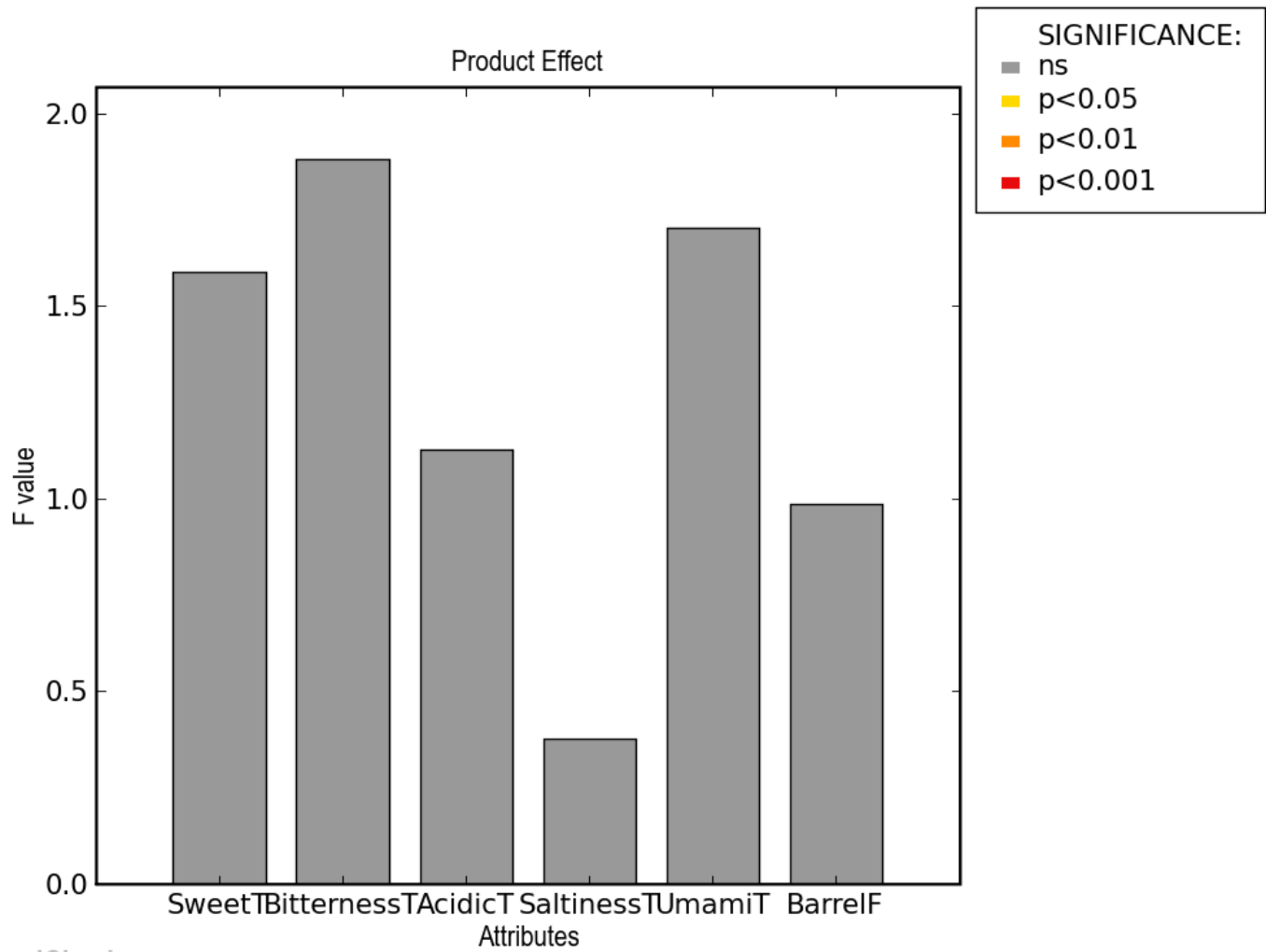
Figure 15: Compiled results from the CATA-test showing the perceived texture and complexity of the samples.

A-7: PanelCheck™ statistical analysis plots



PanelCheck

Figure 16: The Assessor\*Product Interaction created by PanelCheck from the 9-point scale results. It shows that the assessment of the samples were not dependant on the assessors.



PanelCheck

Figure 17: The Product effect created by PanelCheck from the 9-point scale results. It shows that there were not any significant difference between the products regarding the attributes.

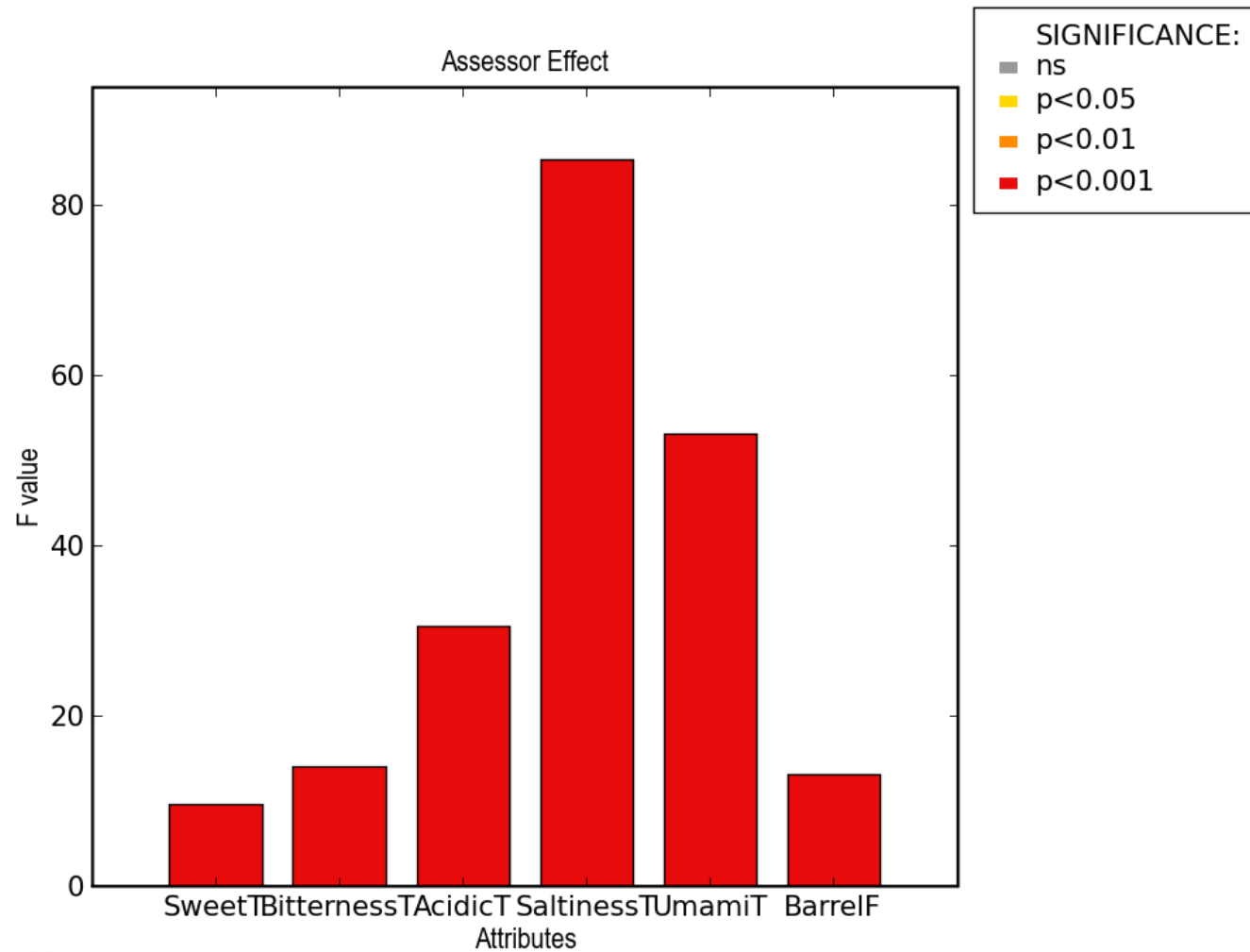


Figure 18: The Assessor effect created by PanelCheck from the 9-point scale results. It shows that there was a significant difference in how the assessors used the scale.

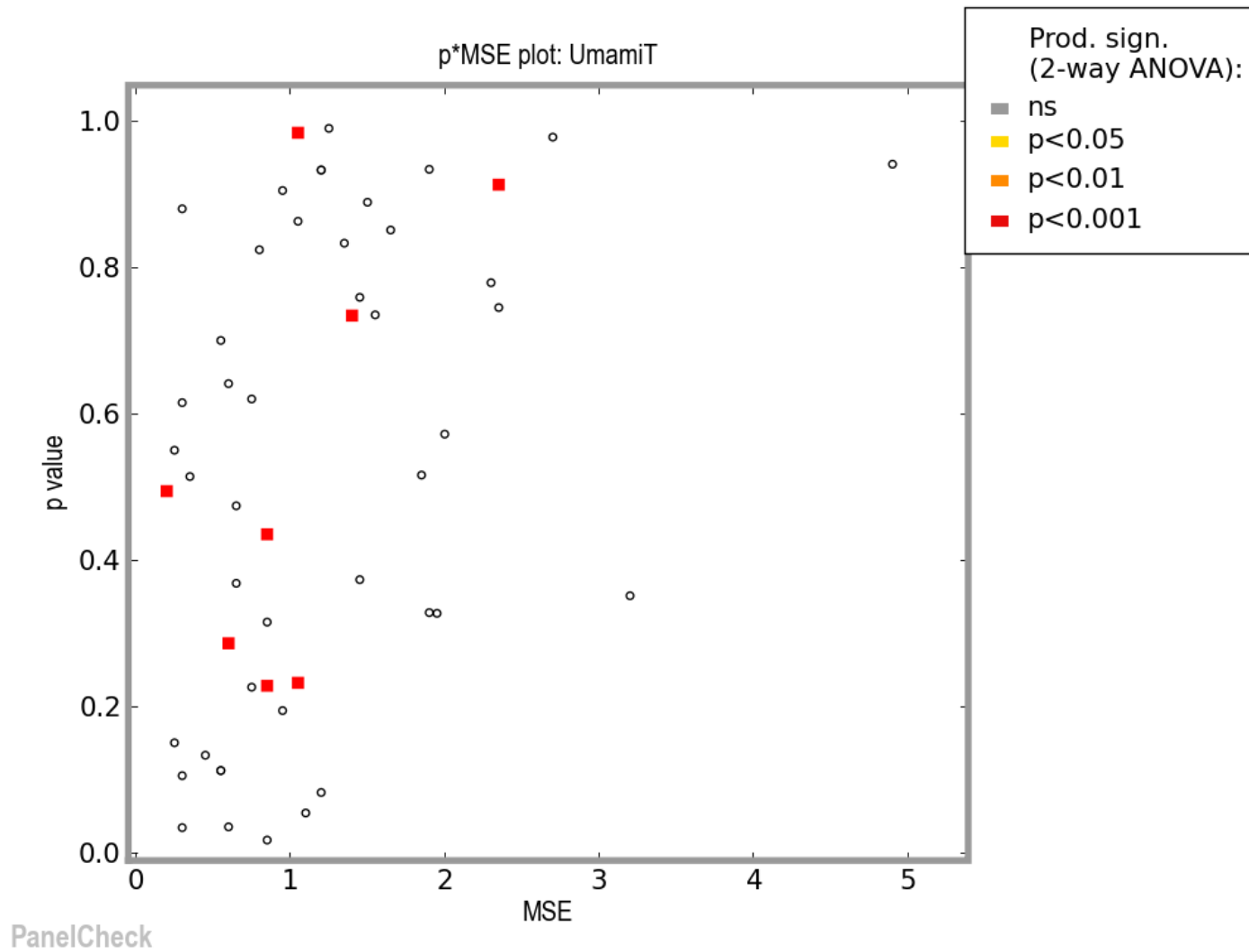
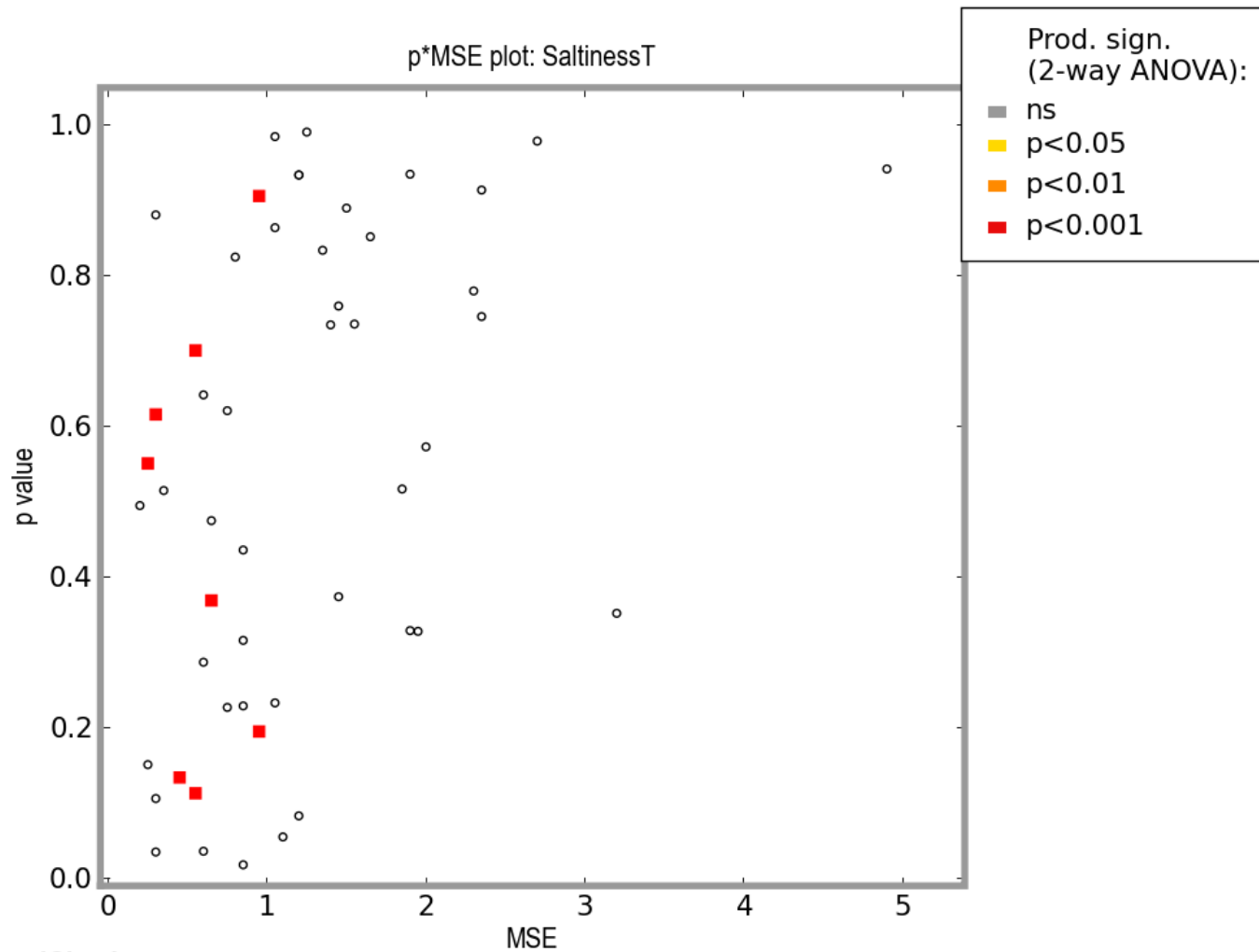


Figure 19: The  $p$ \*MSE plot resulting from a 2-way ANOVA in PanelCheck regarding umami taste. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.





PanelCheck

Figure 20: The p\*MSE plot resulting from a 2-way ANOVA in PanelCheck regarding salt taste. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.

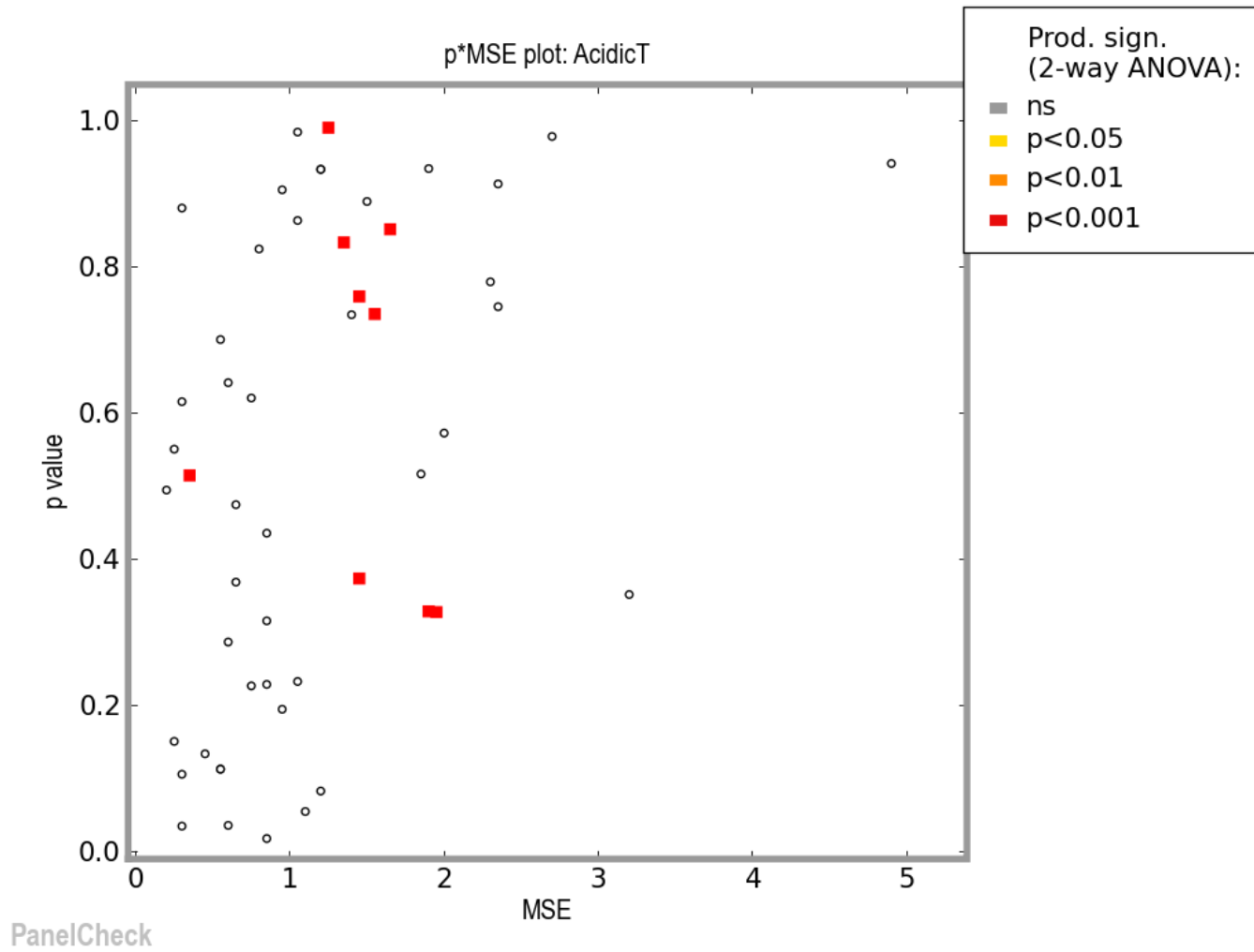


Figure 21: The p\*MSE plot resulting from a 2-way ANOVA in PanelCheck regarding acidic taste. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.

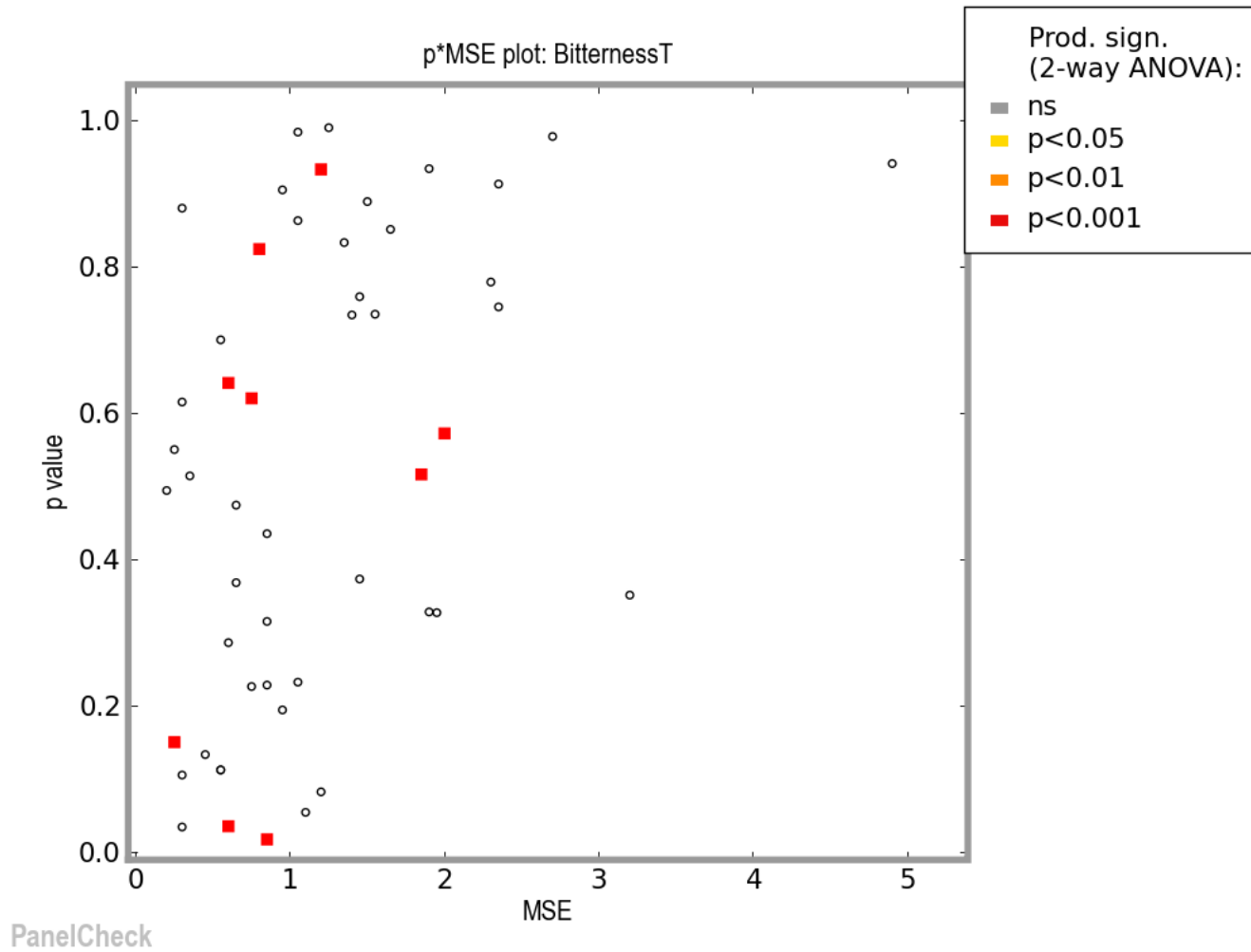


Figure 22: The  $p^*MSE$  plot resulting from a 2-way ANOVA in PanelCheck regarding bitter taste. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.

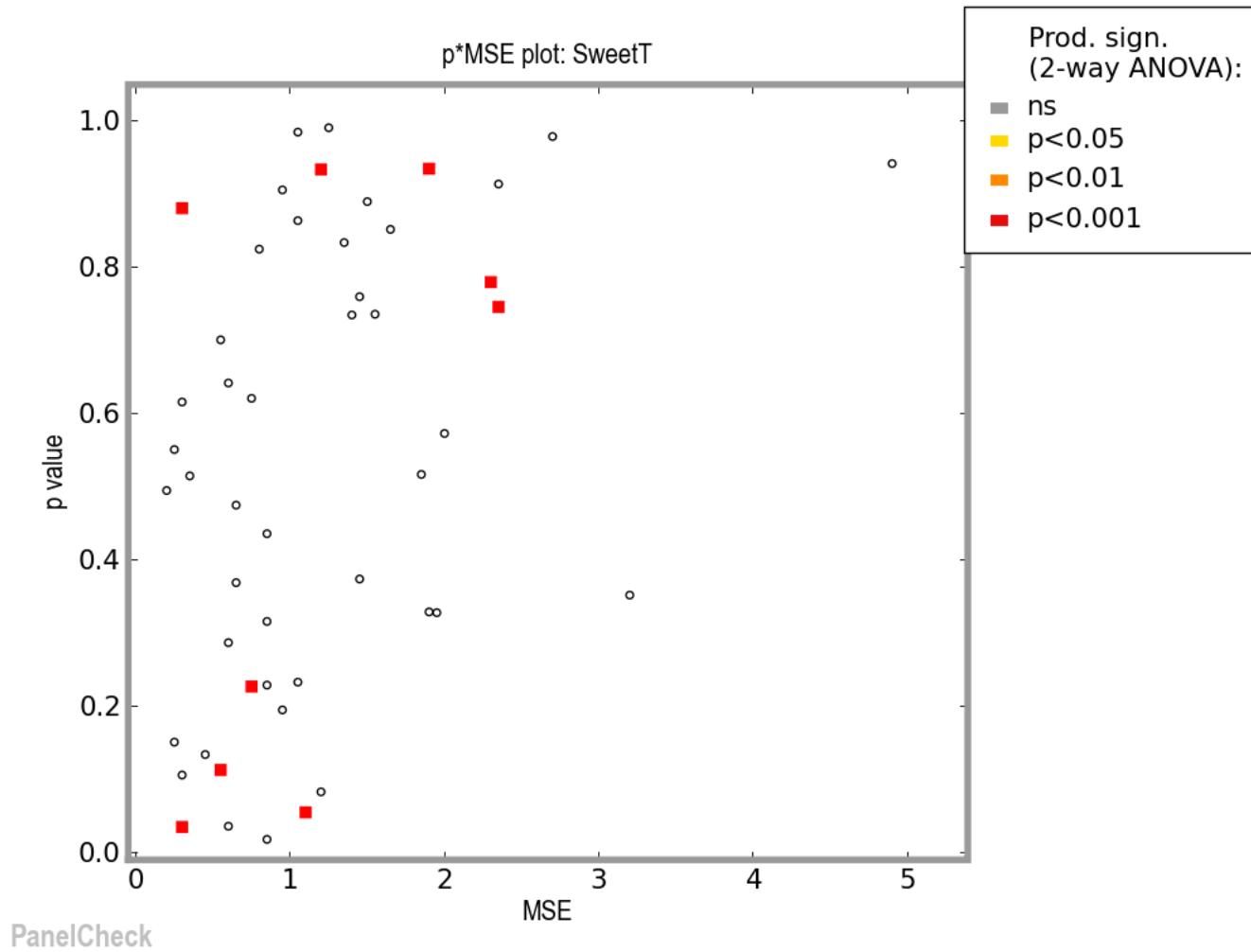


Figure 23: The  $p^*$ MSE plot resulting from a 2-way ANOVA in PanelCheck regarding sweet taste. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.

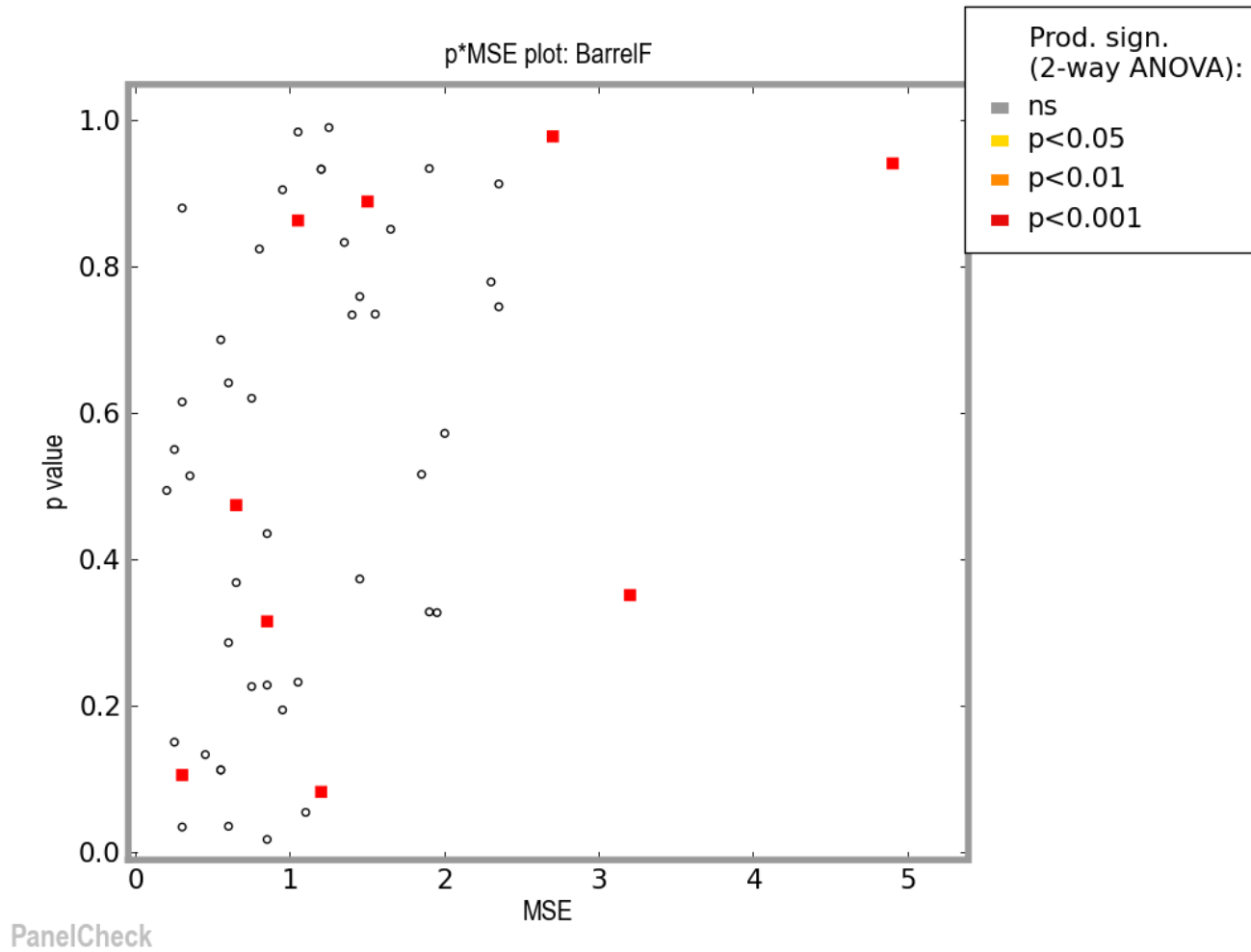


Figure 24: The  $p^*MSE$  plot resulting from a 2-way ANOVA in PanelCheck regarding barrel-related flavour. The red dots represent the compiled scores from the assessors, while the white dots represent the individual datapoints.

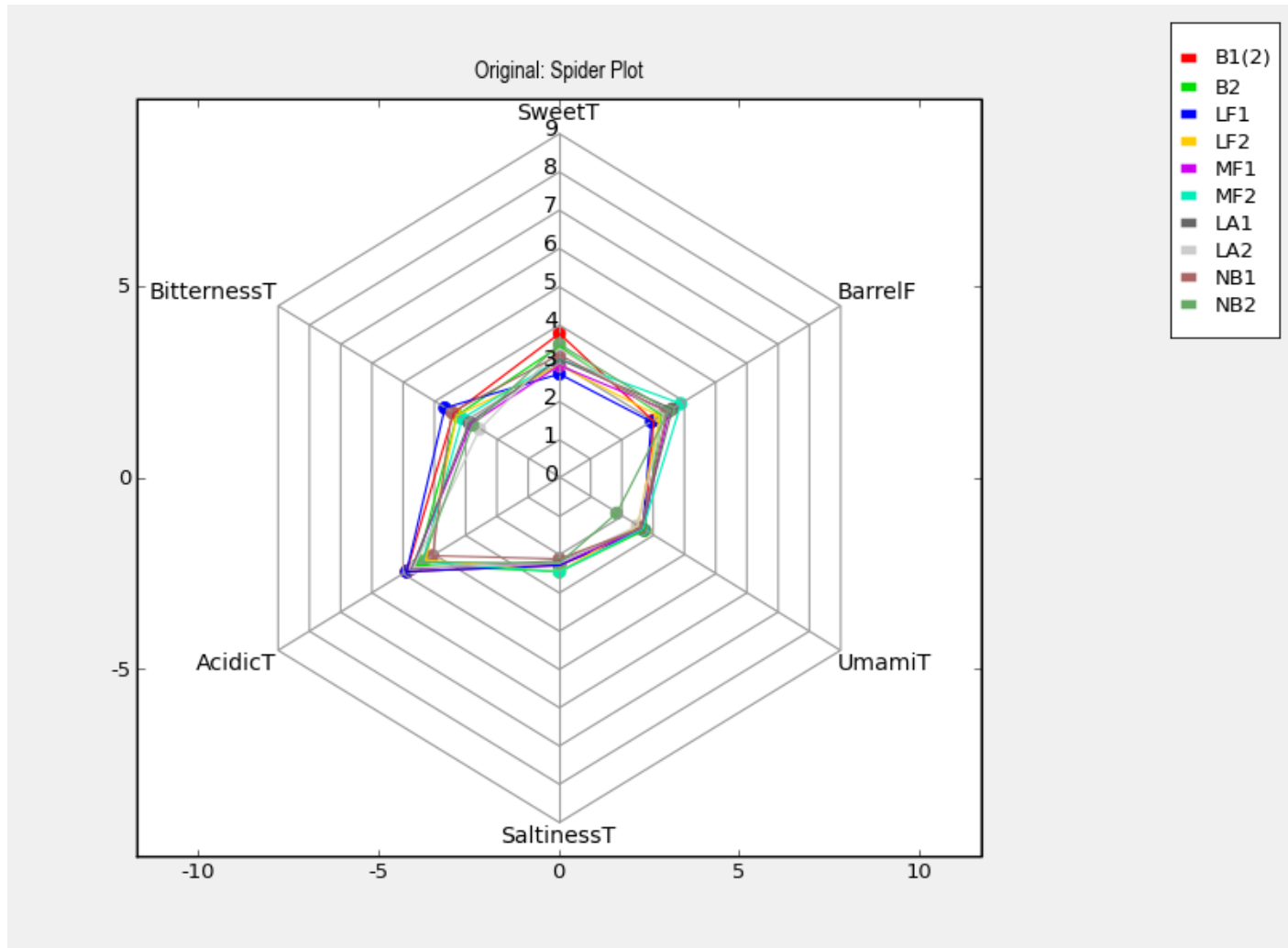


Figure 25: Spider plot compiled from the 9-point scale results by PanelCheck. Each colour represents each product, and each dot represents the score for each respective attribute.

A-8: CATA results: Aroma – Raw data

Table 8: The raw data of the CATA-test regarding aroma. The survey entries were summarized for each series across all assessors.

Code - Tasting	Sample	Citrus	Solvent	Petroleum	Young/ Fresh oak	Old oak	Vanilla	Malt	Alcohol	Cherry	Spice	Herbal	Smoke	Tannins	Orange	Coffee	Chocolate	Hops	Tropical fruits
556	B1(B2) - First series	4	0	0	2	0	1	3	1	0	1	1	1	0	1	0	1	0	1
876	B1(B2) - Second series	4	2	1	1	0	1	5	0	0	2	2	1	0	0	1	0	1	2
126	B2 - First series	1	1	2	1	0	1	2	1	0	5	3	3	0	2	1	0	2	1
771	B2 - Second series	5	0	2	0	1	1	5	0	0	0	3	2	0	1	0	0	3	2
103	LF1 - First series	5	0	2	0	1	1	5	0	0	0	3	2	0	1	0	0	3	2
827	LF1 - Second series	3	1	1	0	1	1	5	1	0	0	1	1	0	0	1	0	1	1
893	LF2 - First series	2	3	0	0	1	3	6	0	0	1	2	1	0	0	0	0	4	2
797	LF2 - Second series	3	1	1	1	1	1	7	2	0	0	1	0	0	1	0	0	2	3
799	MF1 - First series	4	1	0	1	2	1	5	0	0	0	2	2	0	0	0	1	1	1

Code - Tasting	Sample	Citrus	Solvent	Petroleum	Young/Fresh oak	Old oak	Vanilla	Malts	Alcohol	Cherry	Spice	Herbal	Smoke	Tannins	Orange	Coffee	Chocolate	Hops	Tropical fruits
433	MF1 - Second series	3	0	1	1	3	4	5	0	0	0	1	1	0	1	0	0	1	1
244	MF2 - First series	2	0	1	2	2	2	9	0	0	0	3	2	0	1	1	0	1	1
753	MF2 - Second series	5	1	0	1	1	2	5	0	0	0	2	4	0	1	0	0	3	0
184	LA1 - First series	4	1	1	2	3	1	2	1	0	2	1	2	0	2	0	1	1	0
450	LA1 - Second series	4	2	1	1	0	0	2	0	0	1	1	1	0	0	1	0	1	2
614	LA2 - First series	2	1	0	1	1	4	4	0	0	0	0	2	0	2	0	0	1	1
380	LA2 - Second series	4	0	1	2	2	2	4	0	0	0	1	0	0	3	0	0	3	2
954	NB1 - First series	3	4	1	1	2	2	4	2	0	1	2	1	0	1	0	1	1	1
836	NB1 - Second series	3	2	2	1	1	2	3	1	0	1	0	0	0	2	0	1	2	0
294	NB2 - First series	5	0	1	0	1	4	5	1	0	1	0	1	0	0	0	1	1	0



Code - Tasting	Sample	Citrus	Solvent	Petroleum	Young/Fresh oak	Old oak	Vanilla	Malt	Alcohol	Cherry	Spice	Herbal	Smoke	Tannins	Orange	Coffee	Chocolate	Hops	Tropical fruits
721	NB2 - Second series	3	0	1	0	2	2	6	0	0	0	2	1	0	1	0	1	2	1

A-9: CATA results: Flavour – Raw data

Table 9: The raw data of the CATA-test regarding flavour. The survey entries were summarized for each series across all assessors.

Code - Tasting	Sample	Citrus	Solvent	Petroleum	Young/Fresh oak	Old oak	Vanilla	Malt	Alcohol	Cherry	Spice	Herbal	Smoke	Tannins	Orange	Coffee	Chocolate	Hops	Tropical fruits
556	B1(B2) - First series	6	0	0	1	1	2	3	2	0	0	2	1	1	2	0	1	2	3
876	B1(B2) - Second series	6	0	1	2	1	2	5	3	0	2	1	0	0	2	0	0	1	3
126	B2 - First series	5	0	0	3	0	1	4	2	0	3	0	2	2	2	0	0	4	2
771	B2 - Second series	6	2	0	1	3	2	5	4	0	1	3	1	1	1	0	0	3	5
103	LF1 - First series	6	2	0	1	3	2	5	4	0	1	3	1	1	1	0	0	3	5
827	LF1 - Second series	6	0	1	1	2	2	4	4	0	2	2	1	0	0	0	0	2	4
893	LF2 - First series	2	0	3	1	2	2	4	3	1	2	2	0	1	1	0	0	3	3
797	LF2 - Second series	7	2	1	3	1	1	4	2	0	1	2	1	2	0	0	0	3	3
799	MF1 - First series	7	1	1	0	3	4	5	2	0	2	0	3	0	3	0	1	1	2
433	MF1 - Second series	5	2	1	1	2	3	5	3	0	1	2	1	0	1	0	0	3	5

Code - Tasting	Sample	Citrus	Solvent	Petroleum	Young/Fresh oak	Old oak	Vanilla	Malt	Alcohol	Cherry	Spice	Herbal	Smoke	Tannins	Orange	Coffee	Chocolate	Hops	Tropical fruits
244	MF2 - First series	6	2	2	3	1	1	4	2	0	2	3	3	0	1	2	0	2	2
753	MF2 - Second series	5	2	2	3	1	3	4	2	0	3	3	2	1	1	0	0	3	5
184	LA1 - First series	6	2	0	3	2	1	5	2	0	2	3	2	3	2	1	0	3	2
450	LA1 - Second series	5	2	2	1	1	0	3	2	0	1	2	0	1	2	0	0	3	3
614	LA2 - First series	7	2	1	4	1	5	4	2	0	2	2	2	1	1	0	0	3	2
380	LA2 - Second series	6	0	2	4	1	2	4	4	0	0	2	1	1	4	0	1	2	3
954	NB1 - First series	4	4	1	2	1	1	3	4	0	1	3	3	1	1	0	0	1	3
836	NB1 - Second series	4	3	2	4	0	0	1	4	0	2	2	3	1	4	0	0	3	3
294	NB2 - First series	5	1	1	3	1	2	4	1	1	0	1	2	1	1	0	0	2	3
721	NB2 - Second series	6	2	1	1	2	1	4	2	1	1	2	1	0	2	0	0	4	4

#### A-10: CATA results: Colour – Raw data

Table 10: The raw data of the CATA-test regarding colour. The survey entries were summarized for each series across all assessors.

Code - Tasting	Sample	Light yellow	Golden yellow	Light brown	Golden brown	Dark brown	Black and transparent	Black and opaque
556	B1(B2) - First series	0	6	3	0	0	0	0

Code - Tasting	Sample	Light yellow	Golden yellow	Light brown	Golden brown	Dark brown	Black and transparent	Black and opaque
876	B1(B2) - Second series	1	3	3	2	0	0	0
126	B2 - First series	0	7	2	0	0	0	0
771	B2 - Second series	1	5	2	1	0	0	0
103	LF1 - First series	1	5	2	1	0	0	0
827	LF1 - Second series	1	4	3	1	0	0	0
893	LF2 - First series	0	3	3	3	0	0	0
797	LF2 - Second series	2	2	4	1	0	0	0
799	MF1 - First series	0	6	1	2	0	0	0
433	MF1 - Second series	0	5	3	1	0	0	0
244	MF2 - First series	0	6	1	2	0	0	0
753	MF2 - Second series	0	5	1	3	0	0	0
184	LA1 - First series	0	2	3	4	0	0	0
450	LA1 - Second series	0	5	1	3	0	0	0
614	LA2 - First series	0	5	2	2	0	0	0
380	LA2 - Second series	0	4	4	1	0	0	0

Code - Tasting	Sample	Light yellow	Golden yellow	Light brown	Golden brown	Dark brown	Black and transparent	Black and opaque
954	NB1 - First series	0	3	2	4	0	0	0
836	NB1 - Second series	0	5	1	3	0	0	0
294	NB2 - First series	0	5	2	2	0	0	0
721	NB2 - Second series	0	4	3	1	1	0	0

A-11: CATA results: Texture/Complexity – Raw data

Table 11: The raw data of the CATA-test regarding texture and complexity. The survey entries were summarized for each series across all assessors.

Code - Tasting	Sample	Thin	Full bodied	Average	Creamy	Complex	Simple	Explosive/A lot of taste in the beginning	Nuanced	Tame/Flat	Viscous/sticky
556	B1(B2) - First series	3	1	2	1	1	2	2	1	0	0
876	B1(B2) - Second series	2	0	6	0	0	1	1	2	1	0
126	B2 - First series	1	1	4	0	0	1	3	0	1	0

Code - Tasting	Sample	Thin	Full bodied	Average	Creamy	Complex	Simple	Explosive/A lot of taste in the beginning	Nuanced	Tame/Flat	Viscous/sticky
771	B2 - Second series	1	2	4	1	1	1	0	3	0	0
103	LF1 - First series	1	2	4	1	1	1	0	3	0	0
827	LF1 - Second series	2	0	6	0	0	2	0	1	0	0
893	LF2 - First series	2	0	6	0	1	2	1	1	0	0
797	LF2 - Second series	0	1	5	0	0	1	1	3	1	0
799	MF1 - First series	1	1	3	1	3	1	0	1	1	0
433	MF1 - Second series	2	1	3	0	2	1	2	2	0	0

Code - Tasting	Sample	Thin	Full bodied	Average	Creamy	Complex	Simple	Explosive/A lot of taste in the beginning	Nuanced	Tame/Flat	Viscous/sticky
244	MF2 - First series	0	0	6	1	1	3	2	0	0	0
753	MF2 - Second series	0	3	2	1	5	0	0	2	0	0
184	LA1 - First series	0	0	5	3	2	2	0	1	0	0
450	LA1 - Second series	1	1	4	0	3	1	2	1	0	0
614	LA2 - First series	1	1	3	2	3	0	2	1	0	0
380	LA2 - Second series	2	0	5	0	3	2	0	2	0	0
954	NB1 - First series	0	0	2	0	3	1	3	0	2	0

Code - Tasting	Sample	Thin	Full bodied	Average	Creamy	Complex	Simple	Explosive/A lot of taste in the beginning	Nuanced	Tame/Flat	Viscous/sticky
836	NB1 - Second series	0	0	1	2	3	1	3	1	2	0
294	NB2 - First series	1	2	5	1	1	1	0	3	0	0
721	NB2 - Second series	0	1	5	1	1	2	2	0	1	0

A-12: 9-point scale – raw data

Table 12: The raw data used for the analysis of the 9-point scale results through PanelCheck. These results were compiled into a format that is recognisable for the software.

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
101	B1(2)	1	2	6	5	1	1	1
102	B1(2)	1	2	5	7	2	2	2
103	B1(2)	1	3	3	3	2	1	3
104	B1(2)	1	4	3	3	2	4	6
105	B1(2)	1	5	2	7	1	2	2
106	B1(2)	1	5	5	8	8	7	3
107	B1(2)	1	4	5	4	2	3	4

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
108	B1(2)	1	5	5	6	1	1	3
109	B1(2)	1	3	2	3	1	1	1
101	B1(2)	2	3	2	4	1	1	2
102	B1(2)	2	2	6	8	3	3	3
103	B1(2)	2	5	4	3	3	3	3
104	B1(2)	2	6	2	4	2	4	6
105	B1(2)	2	6	1	6	1	3	2
106	B1(2)	2	2	2	4	7	4	3
107	B1(2)	2	4	3	4	2	5	3
108	B1(2)	2	4	2	6	1	3	5
109	B1(2)	2	3	3	3	1	1	2
101	B2	1	2	5	8	1	1	1
102	B2	1	1	6	8	2	2	1
103	B2	1	4	3	4	2	3	4
104	B2	1	3	2	6	1	4	5
105	B2	1	4	2	6	1	3	3
106	B2	1	3	2	3	7	6	4
107	B2	1	4	3	4	3	5	4
108	B2	1	3	3	6	3	2	4
109	B2	1	4	3	3	1	1	3
101	B2	2	2	2	4	1	1	2
102	B2	2	3	4	7	4	4	2
103	B2	2	2	4	3	3	1	3
104	B2	2	5	3	3	4	5	7
105	B2	2	6	2	4	1	1	2
106	B2	2	2	3	6	7	6	3



Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
107	B2	2	4	5	2	1	2	4
108	B2	2	5	3	3	1	1	3
109	B2	2	5	4	2	1	1	4
101	LF1	1	2	4	5	1	1	3
102	LF1	1	2	8	8	3	4	3
103	LF1	1	5	4	4	3	3	4
104	LF1	1	5	2	3	1	6	6
105	LF1	1	2	6	7	1	1	1
106	LF1	1	2	2	5	3	4	2
107	LF1	1	3	5	3	3	3	4
108	LF1	1	5	3	6	2	3	6
109	LF1	1	2	2	3	1	1	3
101	LF1	2	2	4	5	2	1	2
102	LF1	2	2	5	8	3	3	2
103	LF1	2	2	5	3	2	3	1
104	LF1	2	4	2	6	3	4	4
105	LF1	2	2	4	8	1	1	2
106	LF1	2	1	2	6	7	5	2
107	LF1	2	4	2	3	3	3	3
108	LF1	2	2	4	2	1	1	2
109	LF1	2	2	2	3	1	1	3
101	LF2	1	3	3	5	1	1	3
102	LF2	1	2	7	7	3	5	4
103	LF2	1	4	5	5	3	3	5
104	LF2	1	3	2	4	3	4	4
105	LF2	1	3	3	7	2	2	3

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
106	LF2	1	2	2	3	7	6	5
107	LF2	1	4	3	3	1	2	4
108	LF2	1	4	3	3	1	3	3
109	LF2	1	2	3	3	1	1	1
101	LF2	2	2	1	3	1	1	2
102	LF2	2	2	4	8	2	2	1
103	LF2	2	2	4	2	2	2	3
104	LF2	2	6	4	5	3	3	6
105	LF2	2	3	3	5	2	2	3
106	LF2	2	1	2	4	7	4	3
107	LF2	2	4	5	3	3	2	3
108	LF2	2	3	2	4	1	1	2
109	LF2	2	3	3	3	1	1	3
101	MF1	1	3	1	4	1	1	2
102	MF1	1	1	4	7	2	4	6
103	MF1	1	4	3	4	2	2	3
104	MF1	1	5	2	4	1	4	6
105	MF1	1	2	4	4	2	4	6
106	MF1	1	5	2	6	7	5	2
107	MF1	1	4	4	4	2	2	3
108	MF1	1	5	2	4	1	2	4
109	MF1	1	2	3	3	1	1	3
101	MF1	2	2	2	4	1	1	2
102	MF1	2	2	4	8	2	2	2
103	MF1	2	5	3	2	2	2	5
104	MF1	2	1	3	6	2	4	6

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
105	MF1	2	2	3	7	1	2	2
106	MF1	2	1	2	5	8	7	1
107	MF1	2	2	4	6	3	3	5
108	MF1	2	4	3	5	1	1	2
109	MF1	2	3	2	2	1	1	3
101	MF2	1	2	3	3	1	1	2
102	MF2	1	4	5	9	3	3	4
103	MF2	1	2	2	2	1	1	2
104	MF2	1	2	3	5	3	5	6
105	MF2	1	4	2	5	4	4	7
106	MF2	1	4	3	7	7	6	4
107	MF2	1	3	4	2	2	3	4
108	MF2	1	4	3	4	2	3	2
109	MF2	1	3	3	2	1	1	3
101	MF2	2	2	5	4	1	1	2
102	MF2	2	2	5	6	4	4	4
103	MF2	2	3	4	4	1	1	5
104	MF2	2	4	2	5	1	3	6
105	MF2	2	6	2	4	2	2	5
106	MF2	2	2	2	6	7	6	1
107	MF2	2	3	4	5	2	2	6
108	MF2	2	3	2	4	1	1	5
109	MF2	2	3	2	2	1	1	2
101	LA1	1	3	2	4	1	1	2
102	LA1	1	2	4	8	2	6	6
103	LA1	1	3	2	4	2	1	3

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
104	LA1	1	3	2	5	2	5	5
105	LA1	1	3	1	6	1	2	2
106	LA1	1	3	3	6	7	4	1
107	LA1	1	3	4	5	2	3	6
108	LA1	1	5	3	5	1	2	3
109	LA1	1	2	2	3	1	1	3
101	LA1	2	2	4	4	1	1	2
102	LA1	2	1	6	6	2	2	1
103	LA1	2	5	3	4	2	2	2
104	LA1	2	3	3	5	2	2	7
105	LA1	2	6	1	3	2	4	8
106	LA1	2	2	3	5	7	5	3
107	LA1	2	3	5	4	2	4	5
108	LA1	2	4	2	5	1	1	4
109	LA1	2	3	2	3	1	1	2
101	LA2	1	2	2	5	3	1	3
102	LA2	1	4	5	8	3	3	4
103	LA2	1	3	1	2	1	1	4
104	LA2	1	5	2	4	2	4	4
105	LA2	1	4	1	6	1	2	2
106	LA2	1	4	4	7	7	6	3
107	LA2	1	4	3	4	2	3	4
108	LA2	1	4	4	6	1	2	3
109	LA2	1	2	2	3	1	1	3
101	LA2	2	3	2	3	1	1	2
102	LA2	2	2	5	7	2	2	1

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
103	LA2	2	4	2	4	2	2	3
104	LA2	2	4	1	6	2	3	6
105	LA2	2	4	2	3	2	2	3
106	LA2	2	4	2	5	7	6	4
107	LA2	2	2	3	3	1	4	4
108	LA2	2	3	3	5	1	1	4
109	LA2	2	3	2	3	1	1	3
101	NB1	1	2	3	5	1	1	4
102	NB1	1	1	5	5	5	6	7
103	NB1	1	3	4	4	2	2	4
104	NB1	1	4	1	4	2	4	5
105	NB1	1	4	6	4	1	1	4
106	NB1	1	5	3	5	5	5	1
107	NB1	1	3	3	5	2	2	4
108	NB1	1	4	3	3	1	2	6
109	NB1	1	2	2	1	1	1	3
101	NB1	2	2	2	3	1	3	3
102	NB1	2	1	8	8	3	3	1
103	NB1	2	4	5	3	4	3	3
104	NB1	2	6	2	5	1	4	5
105	NB1	2	6	3	5	1	1	2
106	NB1	2	2	3	5	5	5	1
107	NB1	2	4	4	2	1	2	5
108	NB1	2	3	3	3	1	1	2
109	NB1	2	2	2	3	1	1	2
101	NB2	1	3	3	5	1	1	2

Judge	Sample	Replicate	SweetT	BitternessT	AcidicT	SaltinessT	UmamiT	BarrelF
102	NB2	1	3	3	6	2	2	2
103	NB2	1	5	3	4	1	1	4
104	NB2	1	4	2	5	2	1	5
105	NB2	1	4	2	7	2	1	4
106	NB2	1	4	2	2	6	3	4
107	NB2	1	5	5	3	2	3	4
108	NB2	1	5	3	4	1	1	3
109	NB2	1	3	3	3	1	1	4
101	NB2	2	2	2	4	1	1	2
102	NB2	2	4	4	6	3	3	2
103	NB2	2	2	2	3	1	1	4
104	NB2	2	2	3	7	2	2	6
105	NB2	2	4	3	7	4	1	5
106	NB2	2	2	2	4	7	6	6
107	NB2	2	6	4	4	2	3	2
108	NB2	2	3	2	3	1	1	2
109	NB2	2	2	2	2	1	1	2

## Appendix B – Chromatography

### B-1: HSGC-FID – Raw data

Table 13: The raw data from the HSGC-FID. All values are given as ppm. The highlighted values were highlighted with engineer responsible for the analysis, believing them to be quite high.

Sam ple Id	Acet alde hyde	Acet one	Dim ethy lsulf ide	2- met hyl- prop anal	1- prop anol	Diac etyl	Ethy lacet ate	2- met hyl- 1- prop anol	3- met hyl- buta nal	2- met hyl- buta nal	3- met hyl- 1- buta nol	2- met hyl- 1- buta nol	Isob utyl acet ate	Hex anal	2- hexa nol	Buty l acet ate	1- hexa nol	Isoa myl acet ate	Ethy l hexa noat e	Ethy l hept anoa te	Ethy l octa noat e
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
B00 1	5,54 3	0,11 5	0,00 4	0,01 8	45,7 58	n.d.	26,2	77,7 27	0,01 5	0,08 8	153, 719	42,7 12	0,03 8	n.d.	0,64 5	0,00 8	0,04 7	1,19 4	0,04 6	0,00 1	0,05 6
B00 2	5,54	0,07 5	0,00 4	0,00 8	44,2 84	n.d.	25,0 3	75,6 44	0,01 5	0,09 2	149, 426	42,4 07	0,03 5	n.d.	0,60 8	0,00 8	0,04 7	1,12 1	0,04 4	0,00 1	0,06 1
B11 1	3,51 3	n.d.	0,00 9	0,01 7	45,6 43	n.d.	31,9 36	78,0 46	0,01 1	0,09 4	154, 991	43,5 67	0,05 2	n.d.	0,86 5	0,01 6	0,04 5	1,12 7	0,06 1	0,00 1	0,06 2
B21 1	3,67 6	n.d.	0,00 9	0,01 2	46,4 3	n.d.	31,5 31	79,0 28	0,01 1	0,09 1	157, 461	44,3 35	0,05 2	n.d.	0,83 8	0,01 6	0,05 4	1,09 5	0,06 1	0,00 1	0,07 6
LF1 11	3,75 4	0,03 6	0,00 7	0,02 1	44,7 7	n.d.	29,3 42	76,7 11	0,01 1	0,09 1	153, 125	42,8 75	0,05	n.d.	0,77 1	0,01 9	0,04 4	1,00 5	0,06 1	0,00 1	0,07
LF2 11	3,33 2	0,03 1	0,00 4	0,00 8	45,2	n.d.	20,9 05	76,9 24	0,00 9	0,09	153, 57	43,4 25	0,03 7	n.d.	0,58 3	0,01 6	0,06 5	0,75 4	0,05	0,00 1	0,06 9
MF1 11	3,94 7	0,02 1	0,00 5	0,01 2	47,8 37	n.d.	27,1 28	80,5	0,01 3	0,09 2	160, 808	45,1 36	0,04 4	n.d.	0,71 9	0,01 7	0,04 6	0,93 2	0,05 7	0,00 1	0,07 2
MF2 11	3,81 2	0,05 2	0,00 4	0,00 8	47,3 01	n.d.	23,7 97	80,3 5	0,01 2	0,09 7	159, 181	45,0 71	0,03 8	n.d.	0,66 4	0,01 9	0,05 5	0,88 3	0,06 3	0,00 1	0,06 9
LA1 11	3,92 6	0,03 2	0,00 6	0,01 5	46,6 1	n.d.	28,6 57	78,8 46	0,01 2	0,09 3	156, 657	44,4	0,04 7	n.d.	0,75	0,01 6	0,05 9	0,97 4	0,06 1	0,00 1	0,07 1

Sam ple Id	Acet alde hyde	Acet one	Dim ethy lsulf ide	2- met hyl- prop anal	1- prop anol	Diac etyl	Ethy lacet ate	2- met hyl- 1- prop anol	3- met hyl- buta nal	2- met hyl- buta nal	3- met hyl- 1- buta nol	2- met hyl- 1- buta nol	Isob utyl acet ate	Hex anal	2- hexa nol	Buty l acet ate	1- hexa nol	Isoa myl acet ate	Ethy l hexa noat e	Ethy l hept anoa te	Ethy l octa noat e
LA2 11	3,89 5	0,03 4	0,00 4	0,01	47,5 24	n.d.	24,8 4	81,3 63	0,01 3	0,09	161, 779	45,4 81	0,04 1	n.d.	0,67 5	0,01 8	0,06 4	0,88 3	0,06	0,00 1	0,07 7
NB1 11	4,71 1	0,03 4	0,00 5	0,02 1	45,1 8	n.d.	28,6 69	77,6 12	0,01 7	0,09 6	153, 81	43,1 88	0,04 9	n.d.	0,78 7	0,01 6	0,25 7	1,01 1	0,07 4	0,00 2	0,07 6
NB2 11	8,31 8	0,02	0,01 3	0,03 4	49,4 44	n.d.	42,4 99	83,3 88	0,02 1	0,10 5	163, 732	46,3 08	0,06 7	n.d.	1,04 9	0,01 9	0,36 3	1,32 2	0,08 9	0,00 2	0,07 8
B12 1	4,26 7	0,14 9	0,01 3	0,01	48,0 8	n.d.	35,8 33	81,5 22	0,01 4	0,09 7	160, 741	45,1 36	0,05 5	n.d.	0,92 3	0,04 4	0,04 3	1,05 6	0,08 1	0,00 1	0,09
B22 1	4,07	0,04 8	0,00 8	0,01 9	48,3 52	n.d.	28,1 27	81,3 65	0,01 5	0,09 7	161, 75	45,5 54	0,04 8	n.d.	0,74 6	0,04 5	0,05	0,85 4	0,07 4	0,00 1	0,08 5
LF1 21	9,31 7	0,05 2	0,00 6	0,02 2	45,7 94	n.d.	27,3 84	78,4 72	0,01 7	0,09 3	155, 859	44,0 41	0,04 4	n.d.	0,71	0,04 3	0,05 2	0,82 3	0,06 8	0,00 1	0,07 9
LF2 21	46,9 84	0,10 2	0,00 4	0,07 7	45,0 13	n.d.	25,9 99	76,6 02	0,02 6	0,11 1	153, 987	43,8 67	0,04 1	n.d.	0,67 8	0,03 6	0,05 7	0,82 8	0,06 4	0,00 1	0,08 7
MF1 21	23,2 08	0,15 2	0,00 8	0,03 8	44,9 92	n.d.	31,4 86	77,2 72	0,02 3	0,09 9	153, 781	43,2 76	0,04 9	n.d.	0,81 1	0,04 1	0,04 5	0,96	0,07 5	0,00 1	0,09 2
MF2 21	4,88 3	0,03 7	0,00 6	0,00 9	44,4 25	n.d.	27,5 21	76,5 37	0,08	0,08	152, 145	42,8 13	0,04 3	n.d.	0,70 9	0,04 8	0,04 4	0,81 8	0,08 2	0,00 1	0,08 4
LA1 21	6,62 3	0,06 5	0,00 5	0,02 3	47,6 52	n.d.	26,5 41	80,9 58	0,01 4	0,09 2	160, 619	45,5 68	0,04 4	n.d.	0,7	0,05 6	0,07 2	0,80 5	0,07 7	0,00 1	0,09 2
LA2 21	6,66 8	0,05	0,00 7	0,01 3	48,0 58	n.d.	32,9 75	82,1 97	0,01 4	0,09 2	162, 874	45,9 95	0,05 4	n.d.	0,83 7	0,05	0,07 3	0,95 6	0,08 1	0,00 1	0,08 7
NB1 21	24,3 86	0,04 8	0,00 4	0,04 5	44,5 45	n.d.	24,1 51	76,5 58	0,02 8	0,10 3	153, 404	43,3 8	0,03 8	n.d.	0,66 7	0,05 7	0,27 4	0,76 5	0,09 1	0,00 2	0,09 7
NB2 21	29,5 58	0,06 1	0,01 4	0,05	47,2 45	n.d.	43,0 91	80,6 25	0,03 1	0,10 6	159, 021	45,1 21	0,06 6	n.d.	1,02 9	0,06 3	0,39	1,14 6	0,10 6	0,00 2	0,08 7



Sam ple Id	Acet alde hyde	Acet one	Dim ethy lsulf ide	2- met hyl- prop anal	1- prop anol	Diac etyl	Ethy lacet ate	2- met hyl- 1- prop anol	3- met hyl- buta nal	2- met hyl- buta nal	3- met hyl- 1- buta nol	2- met hyl- 1- buta nol	Isob utyl acet ate	Hex anal	2- hexa nol	Buty l acet ate	1- hexa nol	Isoa myl acet ate	Ethy l hexa noat e	Ethy l hept anoa te	Ethy l octa noat e
B14 1	3,42 3	0,03 2	0,01 3	0,01 3	47,5 14	n.d.	33,3 46	80,3 44	0,01 1	0,09	159, 601	44,8 72	0,04 8	n.d.	0,78 8	0,19 3	0,04 7	0,75 9	0,07 6	0,00 1	0,09 3
B24 1	3,59 5	0,05 5	0,01 1	0,01 7	47,5 52	n.d.	29,8 04	80,9 25	0,01 1	0,09 4	160, 391	45,1 13	0,04 4	n.d.	0,71 3	0,17 6	0,04 9	0,68	0,07 3	0,00 1	0,09 2
LF1 41	46,1 28	0,12 4	0,00 9	0,04 7	46,7 72	n.d.	33,5 49	79,6 26	0,02 4	0,10 1	159, 923	44,4 14	0,04 8	n.d.	0,79	0,16 4	0,05 4	0,79 1	0,07 5	0,00 1	0,08 8
LF2 41	57,8 2	0,14 5	0,00 6	0,06 9	44,9 51	n.d.	26,2 29	76,5 07	0,03 1	0,10 7	156, 475	43,7 31	0,03 8	n.d.	0,60 9	0,12 8	0,05 1	0,62 5	0,06 6	0,00 1	0,08 6
MF1 41	48,4 76	0,18 3	0,00 5	0,04 4	41,8 63	n.d.	21,0 45	70,8 5	0,02 2	0,09 1	145, 433	40,6 32	0,03 1	n.d.	0,50 6	0,12 4	0,04 8	0,52	0,05 6	0,00 1	0,07 2
MF2 41	33,5 69	0,07 7	0,00 5	0,03 2	48,3 46	n.d.	29,4 2	80,6 6	0,02 3	0,10 2	162, 138	45,5 76	0,04 1	n.d.	0,67 5	0,19 1	0,05 4	0,66 1	0,07 4	0,00 1	0,07 7
LA1 41	49,8 4	0,25	0,00 8	0,05 0,05	49,4 96	n.d.	30,6 45	82,9 16	0,02 7	0,11	166, 511	47,2 49	0,04 3	n.d.	0,73	0,19 5	0,05 8	0,70 6	0,07 6	0,00 1	0,08 5
LA2 41	35,2 46	0,06 3	0,00 4	0,02 9	47,7 66	n.d.	26,3 39	80,6 55	0,02 1	0,09 7	162, 273	45,5 63	0,03 7	n.d.	0,62 7	0,18 1	0,05 7	0,61 4	0,06 8	0,00 1	0,08 3
NB1 41	145, 006	0,20 5	0,00 5	0,07 6	47,0 18	n.d.	32,3 3	79,4 52	0,02 5	0,10 8	167, 662	46,3 13	0,04 9	n.d.	0,73 9	0,17 4	0,32 7	0,70 2	0,08 3	0,00 2	0,08 2
NB2 41	45,3 55	0,16 3	0,01 3	0,04 9	45,3 46	n.d.	32,8 72	78,0 48	0,03 2	0,10 4	156, 994	43,8 88	0,05	n.d.	0,80 5	0,17 1	0,39 2	0,79 8	0,09 9	0,00 2	0,08 1
B16 1	6,87 5	0,05 7	0,01 7	0,00 4	47,4 85	n.d.	45,0 85	80,9 16	0,01 4	0,09 4	158, 898	44,7 54	0,05 7	n.d.	0,93	0,36 6	0,05 9	0,76 8	0,08 1	0,00 1	0,08 8
B26 1	8,00 3	0,04 7	0,01 7	0,01 2	44,9 15	n.d.	41,8 41	77,6 2	0,01 7	0,08 8	152, 52	42,9 78	0,05 4	n.d.	0,85 1	0,33 8	0,05 3	0,69 3	0,07 6	0,00 1	0,07 6
LF1 61	102, 924	0,14 5	0,00 7	0,07	47,4 31	n.d.	40,5 44	80,1 65	0,02 5	0,10 7	165, 726	46,5 29	0,05	n.d.	0,79 4	0,29 9	0,06 1	0,69 2	0,06 8	0,00 1	0,07 8

Sam ple Id	Acet alde hyde	Acet one	Dim ethy lsulf ide	2- met hyl- prop anal	1- prop anol	Diac etyl	Ethy lacet ate	2- met hyl- 1- prop anol	3- met hyl- buta nal	2- met hyl- buta nal	3- met hyl- 1- buta nol	2- met hyl- 1- buta nol	Isob utyl acet ate	Hex anal	2- hexa nol	Buty l acet ate	1- hexa nol	Isoa myl acet ate	Ethy l hexa noat e	Ethy l hept anoa te	Ethy l octa noat e
LF2 61	111, 585	0,24 5	0,00 5	0,1 0,1	46,8 91	n.d.	32,1 34	78,9 55	0,03 2	0,01 6	165, 649	45,1 73	0,04 3	n.d.	0,65 8	0,25 3	0,07	0,58 5	0,06 5	0,00 1	0,07 1
MF1 61	108, 818	0,19 5	0,00 2	0,08 1	46,6 58	n.d.	27,0 58	77,7 87	0,02 4	0,10 7	163, 527	44,9 42	0,03 5	n.d.	0,56 1	0,25 6	0,06 5	0,49 5	0,06 2	0,00 1	0,07 3
MF2 61	103, 018	0,25	0,00 5	0,08 5	47,2 84	n.d.	32,3 28	79,3 79	0,03 2	0,11 4	165, 022	45,3 17	0,04 1	n.d.	0,62 9	0,30 9	0,06 9	0,55 2	0,06 6	0,00 1	0,06 9
LA1 61	129, 929	0,33 5	0,01	0,10 1	47,0 11	n.d.	38,6 52	78,9 91	0,03 9	0,11 5	166, 101	46,2 85	0,05	n.d.	0,79 1	0,34 3	0,06 1	0,65 2	0,07 3	0,00 1	0,07 6
LA2 61	99,8 6	0,14 3	0,00 6	0,06 4	47,2 29	n.d.	34,7 81	80,2 04	0,02 5	0,10 7	166, 699	45,9 39	0,04 5	n.d.	0,72 9	0,33 1	0,06 4	0,62 3	0,06 8	0,00 1	0,08 3
NB1 61	252, 923	0,54 1	0,00 9	0,11 0,11	47,3 13	n.d.	34,0 78	78,8 16	0,01 5	0,12 8	180, 446	45,6 49	0,04 6	n.d.	0,70 5	0,32 0,32	0,28 4	0,58 7	0,08 3	0,00 2	0,06 4
NB2 61	50,1 22	0,14 8	0,01 2	0,05 5	47,3 16	n.d.	43,0 68	80,0 35	0,03 4	0,10 5	160, 877	44,9 24	0,05 5	n.d.	0,88 7	0,33 1	0,39 8	0,73 6	0,09 3	0,00 2	0,07

## Appendix C – Folin’s method

### C-1: Abs<sub>765</sub> – stock solutions – raw data

Table 14: The calculations and measurements at Abs<sub>765</sub> for the standardization curve used for Folin's method.

Dosage from stock (1g/L)	Standard C (mg/L)	Abs765 1	Abs765 2	Abs765 3	Avg Abs765
0,025 ml	25	0,13	0,128	0,128	0,128667
0,05 ml	50	0,231	0,231	0,231	0,231
0,1 ml	100	0,736	0,748	0,749	0,744333
0,15 ml	150	1,252	1,263	1,266	1,260333
0,2 ml	200	1,73	1,742	1,741	1,737667

### C-2: Standard curve

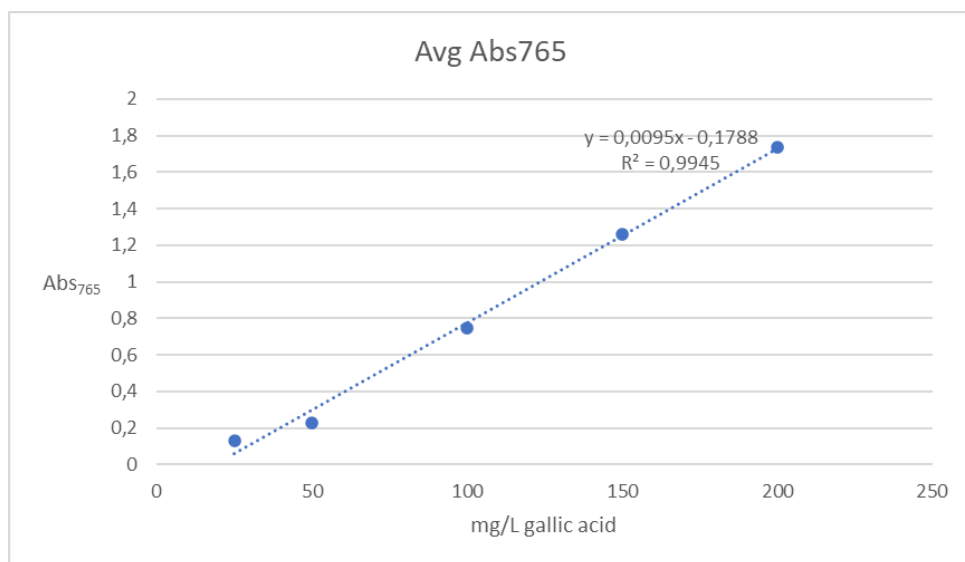


Figure 26: The standard curve for gallic acid created from the measurements in Table 13. The values used were the average Abs<sub>765</sub>.

C-3: Abs<sub>765</sub> and converted data – samples – compiled data

Table 15: The compiled Abs<sub>765</sub> data and their conversions back into the mg GEA/100 mL of the original samples.

Sample	Abs <sub>765</sub> 1	Abs <sub>765</sub> 2	Abs <sub>765</sub> 3	Avg Abs <sub>765</sub> 5	c-1 1:20 (mg GEA/100mL)	c-2 1:20 (mg GEA/100mL)	c-3 1:20 (mg GEA/100mL)	Avg. c (mg GEA/100mL)	Std.D Abs <sub>765</sub>	Std.D c	Org. c-1	Org. c-2	Org. c-3	Avg. Org. c	Std.D. Org. c
B001	0,363	0,363	0,365	0,364	57,03	57,03	57,24	57,10	0,0012	0,1215	114,063	114,063	114,484	1142,04	2,4309
B002	0,373	0,373	0,373	0,373	58,08	58,08	58,08	58,08	0,0000	0,0000	116,168	116,168	116,168	1161,68	0,0000
B111	0,397	0,396	0,396	0,396	60,61	60,51	60,51	60,54	0,0006	0,0608	121,221	121,011	121,011	1210,81	1,2155
B121	0,378	0,379	0,382	0,380	58,61	58,72	59,03	58,79	0,0021	0,2191	117,221	117,432	118,063	1175,72	4,3825
B141	0,400	0,373	0,373	0,382	60,93	58,08	58,08	59,03	0,0156	1,6409	121,853	116,168	116,168	1180,63	32,8178
B161	0,375	0,376	0,376	0,376	58,29	58,40	58,40	58,36	0,0006	0,0608	116,589	116,800	116,800	1167,30	1,2155
B211	0,397	0,400	0,398	0,398	60,61	60,93	60,72	60,75	0,0015	0,1608	121,221	121,853	121,432	1215,02	3,2158
B221	0,366	0,365	0,365	0,365	57,35	57,24	57,24	57,28	0,0006	0,0608	114,695	114,484	114,484	1145,54	1,2155
B241	0,376	0,375	0,375	0,375	58,40	58,29	58,29	58,33	0,0006	0,0608	116,800	116,589	116,589	1166,60	1,2155
B261	0,375	0,376	0,376	0,376	58,29	58,40	58,40	58,36	0,0006	0,0608	116,589	116,800	116,800	1167,30	1,2155
LF111	0,432	0,436	0,435	0,434	64,29	64,72	64,61	64,54	0,0021	0,2191	128,589	129,432	129,221	1290,81	4,3825
LF121	0,386	0,386	0,387	0,386	59,45	59,45	59,56	59,49	0,0006	0,0608	118,905	118,905	119,116	1189,75	1,2155
LF141	0,359	0,359	0,357	0,358	56,61	56,61	56,40	56,54	0,0012	0,1215	113,221	113,221	112,800	1130,81	2,4309

Sample	Avg				c-1 1:20 (mg GEA/100mL)	c-2 1:20 (mg GEA/100mL)	c-3 1:20 (mg GEA/100mL)	Avg. c (mg GEA/100mL)	Std.D Abs765	Std.D c	Org. c-1	Org. c-2	Org. c-3	Avg. Org. c	Std.D. Org. c
	Abs765 1	Abs765 2	Abs765 3	Abs765 5											
LF161	0,369	0,363	0,362	0,365	57,66	57,03	56,93	57,21	0,0038	0,3985	1153,26	1140,63	1138,53	1144,14	7,9704
LF211	0,383	0,381	0,381	0,382	59,14	58,93	58,93	59,00	0,0012	0,1215	1182,74	1178,53	1178,53	1179,93	2,4309
LF221	0,385	0,385	0,386	0,385	59,35	59,35	59,45	59,38	0,0006	0,0608	1186,95	1186,95	1189,05	1187,65	1,2155
LF241	0,370	0,370	0,375	0,372	57,77	57,77	58,29	57,94	0,0029	0,3039	1155,37	1155,37	1165,89	1158,88	6,0774
LF261	0,352	0,352	0,352	0,352	55,87	55,87	55,87	55,87	0,0000	0,0000	1117,47	1117,47	1117,47	1117,47	0,0000
MF111	0,374	0,373	0,373	0,373	58,19	58,08	58,08	58,12	0,0006	0,0608	1163,79	1161,68	1161,68	1162,39	1,2155
MF121	0,397	0,398	0,399	0,398	60,61	60,72	60,82	60,72	0,0010	0,1053	1212,21	1214,32	1216,42	1214,32	2,1053
MF141	0,359	0,359	0,359	0,359	56,61	56,61	56,61	56,61	0,0000	0,0000	1132,21	1132,21	1132,21	1132,21	0,0000
MF161	0,370	0,371	0,371	0,371	57,77	57,87	57,87	57,84	0,0006	0,0608	1155,37	1157,47	1157,47	1156,77	1,2155
MF211	0,396	0,397	0,397	0,397	60,51	60,61	60,61	60,58	0,0006	0,0608	1210,11	1212,21	1212,21	1211,51	1,2155
MF221	0,382	0,381	0,381	0,381	59,03	58,93	58,93	58,96	0,0006	0,0608	1180,63	1178,53	1178,53	1179,23	1,2155
MF241	0,386	0,386	0,386	0,386	59,35	59,45	59,45	59,42	0,0006	0,0608	1186,95	1189,05	1189,05	1188,35	1,2155
MF261	0,374	0,376	0,376	0,375	58,19	58,40	58,40	58,33	0,0012	0,1215	1163,79	1168,00	1168,00	1166,60	2,4309
LA111	0,388	0,388	0,387	0,388	59,66	59,66	59,56	59,63	0,0006	0,0608	1193,26	1193,26	1191,16	1192,56	1,2155

Sample	Avg				c-1 1:20 (mg GEA/100mL)	c-2 1:20 (mg GEA/100mL)	c-3 1:20 (mg GEA/100mL)	Avg. c (mg GEA/100mL)	Std.D Abs765	Std.D c	Org. c-1	Org. c-2	Org. c-3	Avg. Org. c	Std.D. Org. c
	Abs7 65 1	Abs7 65 2	Abs7 65 3	Abs76 5											
LA 121	0,31	0,31	0,31	0,311	51,56	51,56	51,56	51,56	0,0000	0,00	103	103	103	1031,16	0,0000
LA 141	0,37	0,37	0,37	0,371	57,87	57,87	57,87	57,87	0,0000	0,00	115	115	115	1157,47	0,0000
LA 161	0,38	0,38	0,38	0,381	58,82	59,03	58,93	58,93	0,0010	0,10	117	118	117	1178,64	2,1053
LA 211	0,39	0,39	0,39	0,391	59,98	60,08	59,98	60,01	0,0006	0,06	119	120	119	1200,58	1,2155
LA 221	0,38	0,38	0,39	0,388	59,24	59,66	59,98	59,63	0,0035	0,36	118	119	119	1192,84	7,3934
LA 241	0,36	0,36	0,36	0,368	57,56	57,56	57,56	57,56	0,0000	0,00	115	115	115	1151,16	0,0000
LA 261	0,38	0,39	0,38	0,388	59,03	60,19	59,66	59,63	0,0055	0,57	118	120	119	1192,18	11,5949
NB 111	0,36	0,36	0,36	0,368	57,56	57,56	57,45	57,52	0,0006	0,06	115	115	114	1150,16	1,2155
NB 121	0,38	0,38	0,38	0,382	59,24	58,93	58,93	59,03	0,0017	0,18	118	117	117	1180,84	3,6464
NB 141	0,36	0,36	0,36	0,369	57,66	57,66	57,66	57,66	0,0000	0,00	115	115	115	1153,26	0,0000
NB 161	0,35	0,35	0,35	0,353	55,98	55,87	55,98	55,94	0,0006	0,06	111	111	111	1118,95	1,2155
NB 211	0,35	0,35	0,35	0,356	56,29	56,29	56,19	56,26	0,0006	0,06	112	112	112	1125,89	1,2155
NB 221	0,36	0,36	0,36	0,364	57,24	57,14	57,03	57,14	0,0010	0,10	114	114	114	1142,84	2,1053
NB 241	0,35	0,34	0,34	0,349	55,66	55,45	55,45	55,52	0,0012	0,12	111	110	110	1110,26	2,4309

Sample	Absorbance				c-1 1:20 (mg GEA/100mL)	c-2 1:20 (mg GEA/100mL)	c-3 1:20 (mg GEA/100mL)	Avg. c (mg GEA/100mL)	Std.D Abs765	Std.D c	Org. c-1	Org. c-2	Org. c-3	Avg. Org. c	Std.D. Org. c
	Abs7 65 1	Abs7 65 2	Abs7 65 3	Avg Abs76											
NB 261	0,356	0,355	0,355	0,355	56,29	56,19	56,19	56,22	0,0006	0,08	112,589	112,379	112,379	1124,49	1,2155

C-4: Phenolic content over time – Blank – Diagram

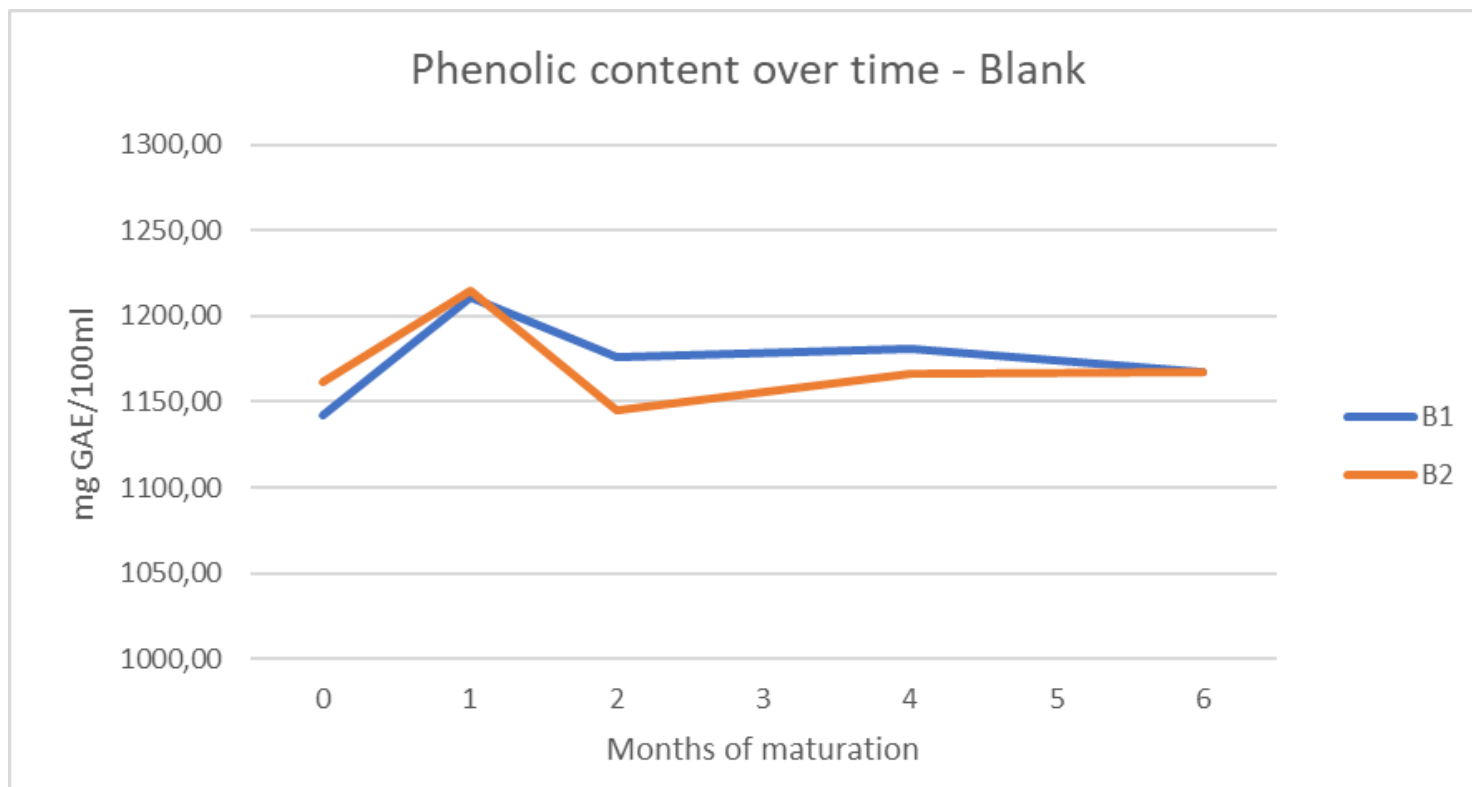


Figure 27: The development of the phenolic content in the Blank samples over the maturation period of 6 months. The graph is made using the average concentrations in the original, undiluted samples.

C-5: Phenolic content over time – Light French oak – Diagram

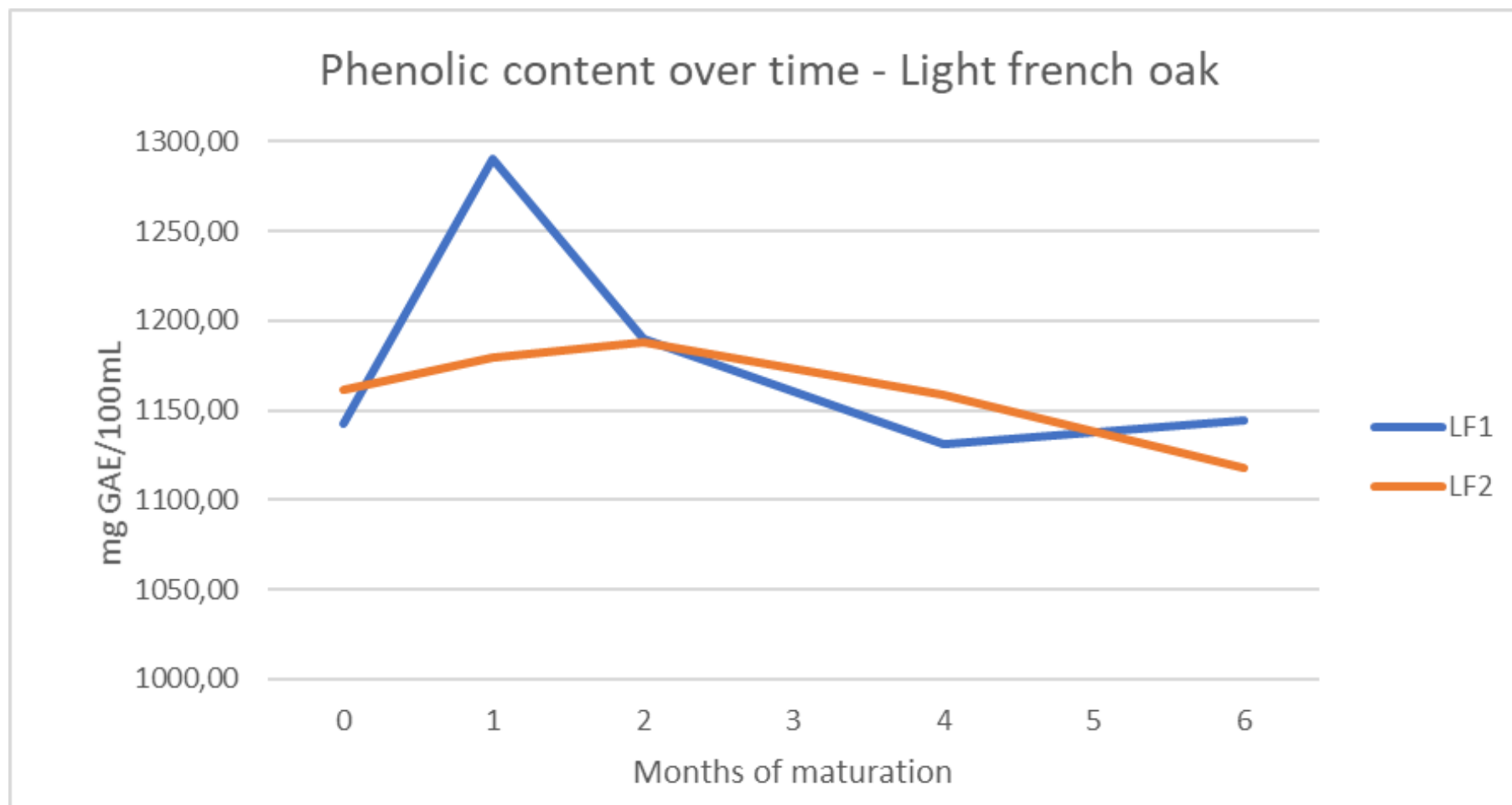


Figure 28: The development of the phenolic content in the Light French oak samples over the maturation period of 6 months. The graph is made using the average concentrations in the original, undiluted samples.



C-6: Phenolic content over time – Medium French oak – Diagram

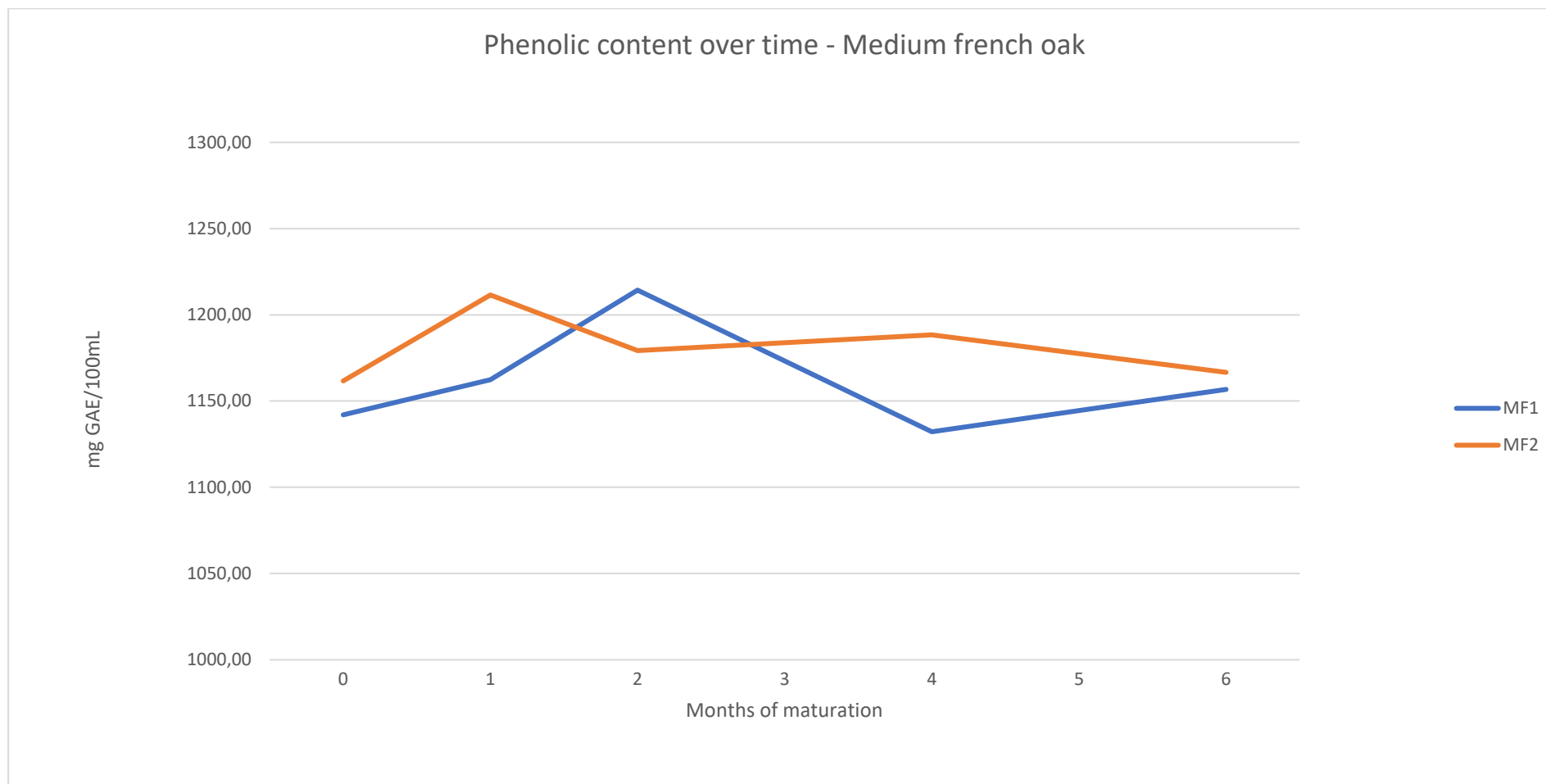


Figure 29: The development of the phenolic content in the Medium French oak samples over the maturation period of 6 months. The graph is made using the average concentrations in the original, undiluted samples.

C-7: Phenolic content over time – Light American oak – Diagram

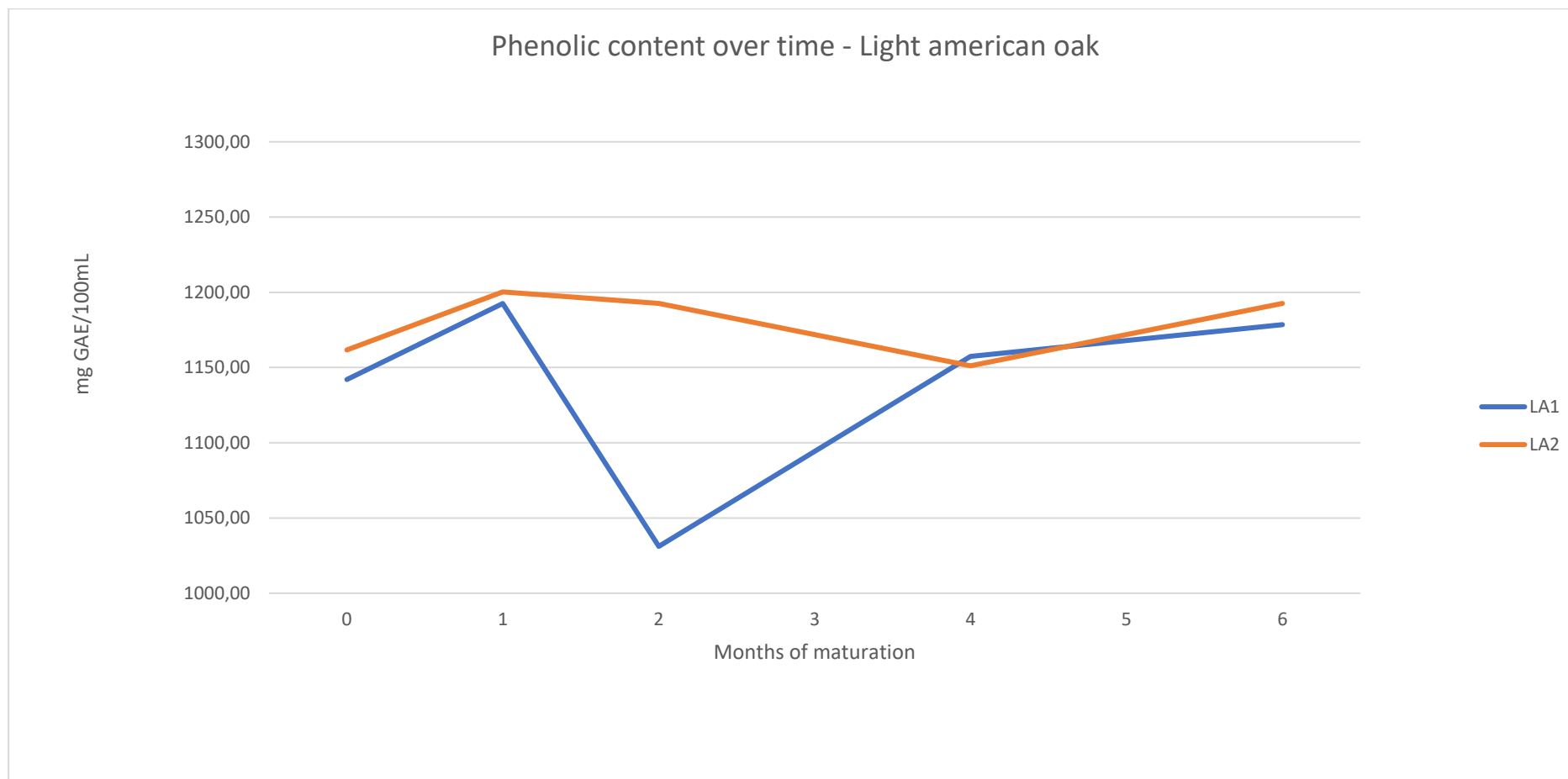


Figure 30: The development of the phenolic content in the Light American oak samples over the maturation period of 6 months. The graph is made using the average concentrations in the original, undiluted samples.

C-8: Phenolic content over time – Norwegian birch – Diagram

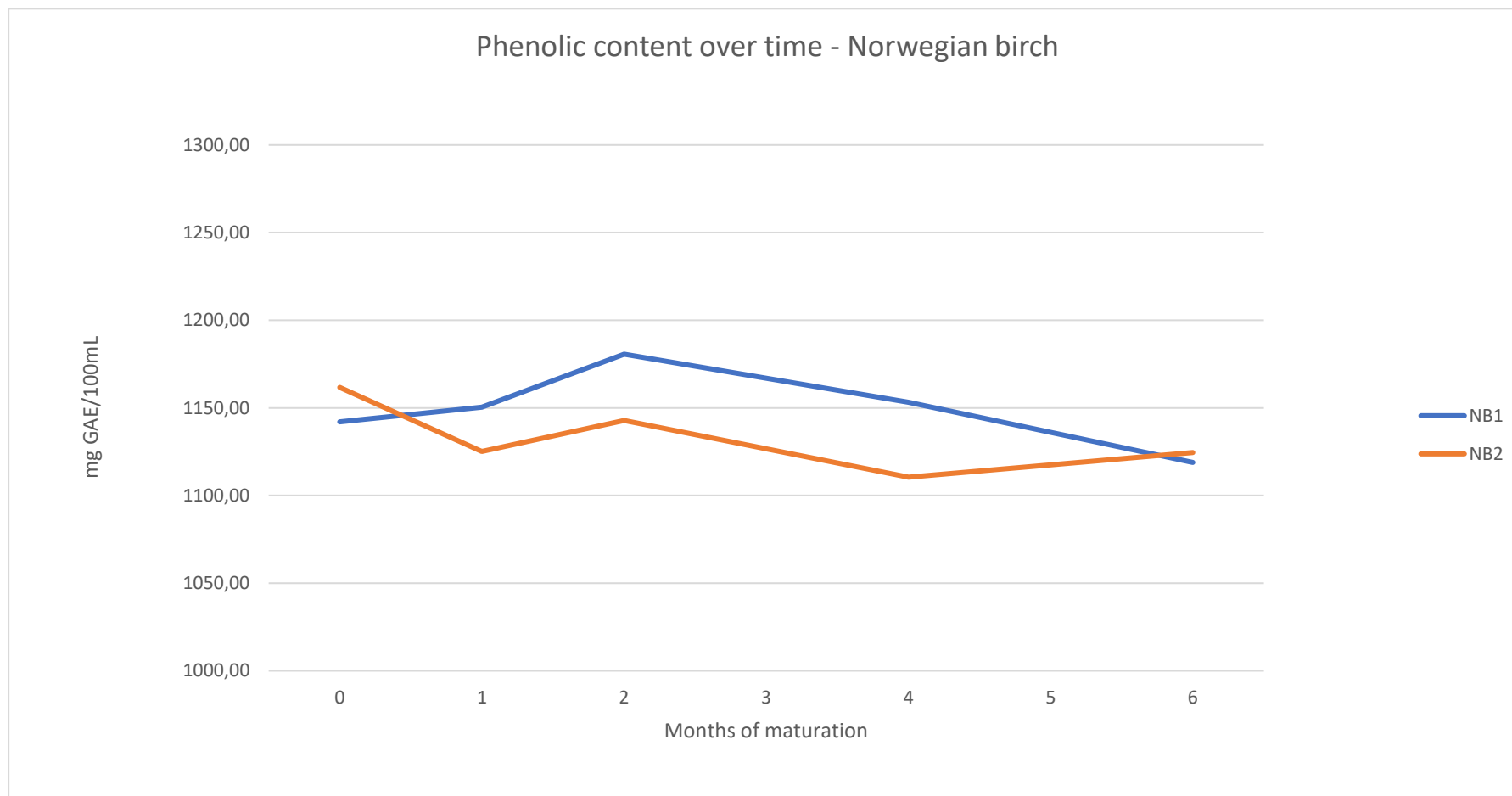


Figure 31: The development of the phenolic content in the Norwegian birch samples over the maturation period of 6 months. The graph is made using the average concentrations in the original, undiluted samples.

## Appendix D – Miscellaneous

### D-1: Codes for extracted samples during maturation

*Table 16: The codes for the samples extracted over the course of the maturation. “X” varies between 1, 2 and 3 to indicate which sample replicate it is.*

<b>Sample source\Months</b>	<b>0</b>	<b>1</b>	<b>2</b>	<b>4</b>	<b>6</b>
B1	B00X	B11X	B12X	B14X	B16X
B2	B00X	B21X	B22X	B24X	B26X
LF1	B00X	LF11X	LF12X	LF14X	LF16X
LF2	B00X	LF21X	LF22X	LF24X	LF26X
MF1	B00X	MF11X	MF12X	MF14X	MF16X
MF2	B00X	MF21X	MF22X	MF24X	MF26X
LA1	B00X	LA11X	LA12X	LA14X	LA16X
LA2	B00X	LA21X	LA22X	LA24X	LA26X
NB1	B00X	NB11X	NB12X	NB14X	NB16X
NB2	B00X	NB21X	NB22X	NB24X	NB26X

### D-2: Codes for HSGC-FID

*Table 17: The codes used for the HSGC-FID samples.*

<b>Sample code</b>	<b>Code HSGC-FID</b>
B001	1
B002	2
B111	3
B211	4
LF111	5
LF211	6
MF111	7
MF211	8
LA111	9
LA211	10
NB111	11
NB211	12
B121	13
B221	14
LF121	15
LF221	16
MF121	17
MF221	18
LA121	19
LA221	20
NB121	21
NB221	22
B141	23

<b>Sample code</b>	<b>Code HSGC-FID</b>
B241	24
LF141	25
LF241	26
MF141	27
MF241	28
LA141	29
LA241	30
NB141	31
NB241	32
B161	33
B261	34
LF161	35
LF261	36
MF161	37
MF261	38
LA161	39
LA261	40
NB161	41
NB261	42

### D-3: Brix, Plato, SG conversion table

Table 18: A Brix, Plato and SG conversion table. Taken from "Straight to the Pint" (Straight to the Pint, 2020).

Brix	Plato	SG	Brix	Plato	SG	Brix	Plato	SG
0.0	0.0000	1.0000	13.4	13.4027	1.0543	26.8	26.7948	1.1140
0.2	0.1970	1.0008	13.6	13.6028	1.0551	27.0	26.9944	1.1150
0.4	0.3970	1.0016	13.8	13.8029	1.0560	27.2	27.1940	1.1159
0.6	0.5970	1.0024	14.0	14.0030	1.0568	27.4	27.3936	1.1168
0.8	0.7970	1.0031	14.2	14.2030	1.0577	27.6	27.5932	1.1178
1.0	0.9970	1.0039	14.4	14.4031	1.0586	27.8	27.7928	1.1187
1.2	1.1970	1.0047	14.6	14.6031	1.0594	28.0	27.9924	1.1197
1.4	1.3971	1.0054	14.8	14.8032	1.0603	28.2	28.1919	1.1206
1.6	1.5971	1.0062	15.0	15.0032	1.0611	28.4	28.3915	1.1216
1.8	1.7971	1.0070	15.2	15.2033	1.0620	28.6	28.5910	1.1225
2.0	1.9972	1.0078	15.4	15.4033	1.0628	28.8	28.7905	1.1235
2.2	2.1972	1.0086	15.6	15.6033	1.0637	29.0	28.9901	1.1244
2.4	2.3973	1.0094	15.8	15.8034	1.0646	29.2	29.1896	1.1254
2.6	2.5973	1.0101	16.0	16.0034	1.0654	29.4	29.3891	1.1263
2.8	2.7974	1.0109	16.2	16.2034	1.0663	29.6	29.5886	1.1273
3.0	2.9975	1.0117	16.4	16.4034	1.0672	29.8	29.7880	1.1282
3.2	3.1975	1.0125	16.6	16.6034	1.0680	30.0	29.9875	1.1292
3.4	3.3976	1.0133	16.8	16.8034	1.0689	30.2	30.1870	1.1302
3.6	3.5977	1.0141	17.0	17.0034	1.0698	30.4	30.3864	1.1311
3.8	3.7977	1.0149	17.2	17.2034	1.0706	30.6	30.5859	1.1321
4.0	3.9978	1.0157	17.4	17.4034	1.0715	30.8	30.7853	1.1330
4.2	4.1979	1.0165	17.6	17.6034	1.0724	31.0	30.9847	1.1340
4.4	4.3980	1.0173	17.8	17.8034	1.0733	31.2	31.1841	1.1350
4.6	4.5981	1.0181	18.0	18.0033	1.0741	31.4	31.3835	1.1359
4.8	4.7982	1.0189	18.2	18.2033	1.0750	31.6	31.5829	1.1369
5.0	4.9983	1.0197	18.4	18.4033	1.0759	31.8	31.7823	1.1379
5.2	5.1984	1.0205	18.6	18.6032	1.0768	32.0	31.9817	1.1389
5.4	5.3985	1.0213	18.8	18.8032	1.0777	32.2	32.1810	1.1398
5.6	5.5986	1.0221	19.0	19.0031	1.0785	32.4	32.3804	1.1408
5.8	5.7987	1.0229	19.2	19.2030	1.0794	32.6	32.5797	1.1418
6.0	5.9988	1.0237	19.4	19.4030	1.0803	32.8	32.7791	1.1428
6.2	6.1989	1.0245	19.6	19.6029	1.0812	33.0	32.9784	1.1437
6.4	6.3990	1.0253	19.8	19.8028	1.0821	33.2	33.1777	1.1447
6.6	6.5991	1.0261	20.0	20.0027	1.0830	33.4	33.3770	1.1457
6.8	6.7992	1.0269	20.2	20.2026	1.0839	33.6	33.5763	1.1467
7.0	6.9994	1.0277	20.4	20.4025	1.0848	33.8	33.7756	1.1477
7.2	7.1995	1.0285	20.6	20.6024	1.0857	34.0	33.9749	1.1487
7.4	7.3996	1.0294	20.8	20.8023	1.0866	34.2	34.1741	1.1497
7.6	7.5997	1.0302	21.0	21.0021	1.0875	34.4	34.3734	1.1507
7.8	7.7998	1.0310	21.2	21.2020	1.0884	34.6	34.5727	1.1516
8.0	7.9999	1.0318	21.4	21.4018	1.0892	34.8	34.7719	1.1526
8.2	8.2000	1.0326	21.6	21.6017	1.0901	35.0	34.9711	1.1536
8.4	8.4002	1.0334	21.8	21.8015	1.0911	35.2	35.1703	1.1546
8.6	8.6003	1.0343	22.0	22.0014	1.0920	35.4	35.3695	1.1556
8.8	8.8004	1.0351	22.2	22.2012	1.0929	35.6	35.5687	1.1566
9.0	9.0005	1.0359	22.4	22.4010	1.0938	35.8	35.7679	1.1576
9.2	9.2006	1.0367	22.6	22.6008	1.0947	36.0	35.9671	1.1586
9.4	9.4007	1.0376	22.8	22.8006	1.0956	36.2	36.1663	1.1596
9.6	9.6009	1.0384	23.0	23.0004	1.0965	36.4	36.3655	1.1606
9.8	9.801	1.0392	23.2	23.2002	1.0974	36.6	36.5646	1.1617
10.0	10.0011	1.0400	23.4	23.4000	1.0983	36.8	36.7638	1.1627
10.2	10.2012	1.0409	23.6	23.5997	1.0992	37.0	36.9629	1.1637
10.4	10.4013	1.0417	23.8	23.7995	1.1001	37.2	37.1620	1.1647
10.6	10.6014	1.0425	24.0	23.9992	1.1011	37.4	37.3612	1.1657
10.8	10.8015	1.0434	24.2	24.1990	1.1020	37.6	37.5603	1.1667
11.0	11.0016	1.0442	24.4	24.3987	1.1029	37.8	37.7594	1.1677
11.2	11.2017	1.0450	24.6	24.5984	1.1038	38.0	37.9585	1.1688
11.4	11.4018	1.0459	24.8	24.7982	1.1047	38.2	38.1576	1.1698
11.6	11.6019	1.0467	25.0	24.9979	1.1057	38.4	38.3566	1.1708
11.8	11.8020	1.0475	25.2	25.1976	1.1066	38.6	38.5557	1.1718
12.0	12.0021	1.0484	25.4	25.3972	1.1075	38.8	38.7548	1.1728
12.2	12.2022	1.0492	25.6	25.5969	1.1084	39.0	38.9538	1.1739
12.4	12.4023	1.0501	25.8	25.7966	1.1094	39.2	39.1529	1.1749
12.6	12.6024	1.0509	26.0	25.9963	1.1103	39.4	39.3519	1.1759
12.8	12.8025	1.0518	26.2	26.1959	1.1112	39.6	39.5509	1.1770
13.0	13.0026	1.0526	26.4	26.3956	1.1122	39.8	39.7500	1.1780
13.2	13.2027	1.0534	26.6	26.5952	1.1131	40.0	39.9490	1.1790

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---

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