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# BEER FLAVOR COMPONENT ANALYSES FOR SMALL BREWERIES

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# PIENPANIMOLLE SOVELTUVAT OLUEN AROMI-AINESTEN ANALYSOINTIMENETELMÄT

**Tiivistelmä**

Oluen teollinen valmistus tukeutuu enenevissä määrin kemiaan ja kemiallisiin menetelmiin vaikkakin oluen panemisella on ikivanhat perinteet. Syy tähän on tämän päivän laatuvaatimuksissa, tuotekehittelyssä ja kilpailussa markkinaosuuksista. Pakollisten analyysien määrä on pieni, mutta uudenaikaiset analyysimenetelmät tuovat mukanaan huikeita mahdollisuuksia tuotekehittelyyn ja laadunvalvontaan. Oluen flavoriaineita on suuri määrä ja jos lisätään siihen lukuun vielä alkuperäiset (esim. humalan) aromiaineet, mahdolliset välimuodot, johdannaiset sekä oluen maulle haitalliset yhdisteet, saadaan erittäin suuri määrä tunnistettavia yhdisteitä. Panimo joutuu siten jossain vaiheessa päättämään haluaako se hyödyntää näitä mahdollisuuksia tuotekehittelyssä tai laadun tarkkailussa. Laboratorion perustaminen on kuitenkin kallista ja vaatii lisäksi hyvää kemian sekä analyysimenetelmien ja -laitteiden tuntemusta ja hallintaa. Tässä tutkielmassa oluen panoa tarkasteltiin lähinnä kemistin näkökulmasta siten että painotus on aromiaineiden karakterisoimisessa sekä niiden määrän ja laadun analysoimisessa.

Laboratorion perustamisen taloussuunnittelu voidaan aloittaa selvittämällä sen hyödyt ja haitat esim. laadun varmistuksen tai panimon talouden kannalta. Kirjallisuuskatsaus osoitti, että oluen tärkeimmät yhdisteet, ovat erilaiset karbonyylit kuten aldehydit, ketonit ja orgaaniset hapot. Muita makuun vaikuttavia komponentteja ovat proteiinit, rikkiä sisältävät yhdisteet, polyfenolit ja liuenneet mineraalit. Näiden yhdisteiden analysoimiseen tarvitaan kallista teknologiaa, joten budjetin tulisi siten olla melko suuri. Investointilaskelmien kolmiarvoiset (realistiset-, optimistiset- ja pessimistiset-) odotuslaskelmat osoittivat että suurenkin investoinnin takaisinmaksuaika on hallittavissa analysointikapasiteettia ja suurimpia kuluja, lähinnä palkkoja, optimoimalla. Selvitys osoitti, että HPLC-laitteiston hankinta voisi olla riittävä, mikäli laboratorio tuottaa analyysejä panimon omiin tarpeisiin. Mikäli tarve syntyy herkemmälle analytiikalle, esim. tarkkoja tietoja raaka-aineista, vierteen aromiaineista, kontaminanteista, jne., on harkittava GC-MS laitteiston hankkimista. Tällöin on syytä tarjota analyysipalveluja kolmansille osapuolille, jotta laboratorion toiminta olisi kannattavaa.

ASIASANAT:

Oluen pano, aromiaine, analyysimenetelmät, haihtuva aine, GC-MS, HPLC, kromatografia, investointianalyysi, kannattavuus

MASTER'S THESIS | ABSTRACT

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## Abstract

Brewing beer is a craft with ancient traditions. Breweries of today, however, must rely increasingly on chemistry in addition to traditions, to meet the demands for quality control, product development and market shares. The minimum number of required chemical analyses are few but the opportunities provided by modern chemical analysis methods are vast. Establishing a laboratory is, however, costly and requires often deep knowledge of chemistry, analysis techniques and equipment. The knowledge of flavor development and transformations of an enormous number of compounds are needed for the laboratory to function successfully. There are some analyses that are obvious, such as the determination of the alcohol (ethanol) content. When the brewing becomes more focused on economic issues, the need for identification of off-flavors, flavor components, and problems in the process planning, increase. The demand for detailed knowledge of the amounts and types of chemical compounds in the raw materials, and the final beer, inevitably grows. In the present study, brewing is thus studied mainly from a chemist´s point of view with the emphasis on the impact of different flavor compounds on beer taste and how to analyze their content.

Economic planning of a laboratory can be started by outlining the advantages and disadvantages of analysis instruments, and what their role in for example quality control could be. A literature survey showed that the most important flavor components, in beer, are carbonyl compounds such as aldehydes, ketones and organic acids. Other important flavor affecting compounds are proteins, sulphur-containing compounds, polyphenols, and solubilized minerals (sulphates and calcium bi-carbonate). The analysis equipment needed to analyze and quantify these compounds must, therefore, be sophisticated enough and the investment should thus be rather large. Tri-valued re-payment time calculations showed that large investments can be made if only the analysis capacity is optimally used and the fixed costs, mostly salaries, are closely controlled. Analyses must thus be sold to third parties to handle the costs. An investment in a HPLC system would suffice for a small brewery, if the analyses were made solely for the brewery's own use. A detailed analysis of raw materials, intermediate products (e.g. malt, samples during fermentation etc.) and the final beer, calls for more potent analysis methods e.g. a GC-MS.

KEYWORDS:

Brewing, flavor components, analysis, volatiles, GC-MS, HPLC, investment analysis

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# USED ABBREVIATIONS AND VOCABULARY

| Abbreviation | Explanations to the used abbreviations or concepts |
|---|---|
| ABC | Activity Based Costing, a method by which costs (resources) are assigned to identified activities and activities to cost objectives (based on consumption approximations) using cost drivers. In that way resource consumption can be traced, and pricing is more accurate. |
| Analogue (chemical) | A compound having a similar structure, to another compound, but that differs regarding a certain component (e.g. one or more atoms, functional groups, or substructures) |
| Cis-trans isomerism | Cis indicates that the functional groups (often denoted "R") are situated on the same side while trans indicates that the substituents are on the opposing side of the carbon chain. Cis-trans (Latin) isomers, also known as geometric or conformational isomers, are stereoisomers. |
| Coke | A solid carbonaceous material derived from pyrolysis (heating at a low oxygen content) of low-ash and low-sulfur bituminous coal |
| Copper | Boiling vessel for the wort |
| Diastace | Starch-degrading enzymes (referred collectively) |
| Diastatic power (DP) | The activity (hydrolytic efficiency) of the diastace in malt |
| DMS | Dimethyl Sulphide, $(CH_3)_2S$, an off-flavor in beer |
| EBC | European Brewery Convention, represents the technical and scientific interests of the brewing sector in Europe |
| FAN | Free Amino Nitrogen |
| FPD | Flame Photometric Detector |
| GC | Gas chromatography |
| GC-MS | Gas chromatography with mass-spectrometric detection |
| Grits | Hard sharp granules |
| Grist | The product obtained from grinding grain |
| Homolytic | Breakdown to equal pieces, i.e. chemical bonds in a neutral molecule dissociate resulting in the generation of free radicals |
| HPLC | High Pressure (Performance) Liquid Chromatography |
| IBU | International Bitterness Units |
| IoB | The Institute of Brewing |

| | |
|---|---|
| Isomer | An isomer is a molecule having the same molecular formula, as another molecule, but with a different chemical structure. The isomerism can be structural or spatial (stereoisomerism). |
| Kiln(ing) | Oven for drying, drying in a specially constructed oven |
| ºL | Degrees Lovibond is a measure of the color of a substance |
| Mash | Mixture of malt and brewing water |
| MBT | 3-methylbut-2-ene-1-thiol, a skunky off-flavor |
| PFPD | Pulsed Flame Photometric Detector |
| SG | Specific Gravity. The relative density, compared to water, of the wort. The SG is measured using a hygrometer, a pycnometer, a refractometer, or a oscillating U-tube electronic meter. |
| SPME | Solid-Phase Micro Extraction |
| SRM | Standard Reference Method is a system to specify beer color |
| TSN | Total soluble nitrogen |
| UV-Vis | The ultraviolet and visible light regions of the spectrum |
| VDK | Vicinal Diketone (also called diacetyle) |
| WACC | Weighted average cost of capital |

# 1 INTRODUCTION

The skill of brewing is more than 8000 years old and it is known that, at least all major early civilizations (e.g. in Mesopotamia, Egypt and Greece) mastered the art of brewing. Although the principles of brewing are simple, the end result depends of how skillful the brewer is, and how well he/she can take advantage of the many possibilities to fine-tune taste and flavor of the beer. Most cultures and countries have their own typical beers. The differences arise primarily from choices of raw materials and brewing methods. There are thus many types of beers differing in taste, color, strength, and composition. Different beer types have their typical color intensities as can be seen from **Table 1**.

**Table 1.** Beer types classified according to their color.

SRM (The Standard reference method), Lovibond (scale devised by J.W. Lovibond, a British brewer) and EBC (Standard Reference Method). Table adopted from https://en.wikipedia.org/wiki/Beer_measurement#Colour

| SRM/Lovibond | Example | Beer color | EBC |
|---|---|---|---|
| 2 | Pale lager, Witbier, Pilsener, Berliner Weisse | | 4 |
| 3 | Maibock, Blonde Ale | | 6 |
| 4 | Weissbier | | 8 |
| 6 | American Pale Ale, India Pale Ale | | 12 |
| 8 | Weissbier, Saison | | 16 |
| 10 | English Bitter, ESB | | 20 |
| 13 | Biere de Garde, Double IPA | | 26 |
| 17 | Dark lager, Vienna lager, Marzen, Amber Ale | | 33 |
| 20 | Brown Ale, Bock, Dunkel, Dunkelweizen | | 39 |
| 24 | Irish Dry Stout, Doppelbock, Porter | | 47 |
| 29 | Stout | | 57 |
| 35 | Foreign Stout, Baltic Porter | | 69 |
| 40+ | Imperial Stout | | 79 |

Beer color can thus also be seen as a measure of quality. The beer quality is traditionally followed using sensory impression where the flavor, aroma, mouthfeel, and color are the most important issues to follow. This has resulted in highly developed brewing methods based on experience and traditions. One example of this is could be the German Beer Purity Law (reinheitsgebot), from 1516, which stipulates that (apart from the price of 1-2 pfennig / ~1,07 L beer) only water, barley, and hops must be used in brewing beer. Modern breweries produce huge volumes (trillions of liters) of beer at minimum time which imposes strict demands on process control and economy.

The necessary analyses (the minimum amount) a brewer needs are only a few. The perhaps most important analysis, already because of the current legislation, is the measurement of the **ethanol content**. Other important analyses are **microbiological** identifications of beer spoiling microbes, and the **oxygen** and **$CO_2$** contents. Other frequently used analyses are pH measurements and assessments of physical appearance, e.g. beer haziness, color, and foam (head) stability. However, to increase the possibilities to a successful business development, the assortment of analysis equipment should be increased so that beer composition can be analyzed. Already measurements of beer bitterness require analysis instruments and qualified personnel to use these apparatuses.

Today's chemistry and, above all, new and developed chemical analysis methods, make it possible to plan chemical reactions, monitor processes and detect unwanted reactions. The concentrations and proportions of the various flavor components form a fingerprint that tells you, immediately, if the flavor is correct. The brewing conditions can be followed by sampling and analyzed at suitable intervals, and if the resulting component proportions are wrong, appropriate measures can be taken. Possible reasons can be elucidated, for example if the brew has been contaminated with poor raw materials or if beer spoiling microorganisms are present.

The acquisition of analysis equipment means often a considerable expense to the company and, especially if the firm has a small turnover, can thus turn out to be a heavy economic burden. All aspects of the purchase must therefore be made and the most common way to do that is to perform an investment analysis. An investment analysis must be made in such a way that it takes into account the economic investment itself, all costs involved in maintaining – and repaying- the investment, and the resulting profit.

# 2 BASICS OF BREWING AND THE MOST IMPORTANT FLAVOR COMPONENTS IN BEER

Beers are made all over the world using a multitude of methods and raw materials. To limit the scope, types of beers and brewing methods, mentioned in the present thesis, are therefore limited to those developed in Europe. Brewing is, simply, to steep a starch source in water releasing sugars and then to ferment this liquid. However, brewing is a much more sophisticated procedure where every step in the process is carefully planned and optimized (**Figure 1**). An apparent key step in beer production is the selection and preparation of good raw materials. The main cereal used for brewing is barley but also wheat, corn, sorghum, rye, and oats are used for making beer.

### 2.1 Malting

Apart from best quality; water, cereal grains, hops and yeast, the **malting** process is crucial for the brewing process and the resulting taste of the beer. Malting starts by activating the dormant grains. This is done by drying the grains to <14% humidity. Germination of the grains is then started by steeping them in brewing process water until they sprout. This process activates enzymes needed for starch (amylases) and protein degradation (proteases) into various sugars and amino acids. The sugars are mostly maltodextrins (higher sugars), maltotriose (trisaccharide), maltose (disaccharide), and glucose (monosaccharide). Proteins are degraded into smaller fragments which are then later used by the yeast. The sprouting is stopped, at a suitable time, by heating the malt up to ~80 - 100° C. The germinated seeds are simultaneously dried in a kiln to a moisture content of 1,5 – 6% (on fresh weight basis). The deactivation of the germination (enzyme activity) can also be made using hot smoke or by roasting. The **kilning** conditions (especially temperature and moisture) may be varied according to process demands. The grains are then ground (dressed) to ease the conversion of starch (release of sugars) during mashing and to remove unwanted parts like rootlets and dust. These treatments give beer special flavors and colors and can be varied according to demand. The produced material is now called **malt** and the quality of this beer raw material is essential for the value of the final product (**Figure 1**). Many malt characteristics, e.g. the nitrogen- (protein) and β-glucan contents must be considered when the barley is malted. Malt properties (and price) are sometimes adjusted using adjuncts (usually 10 – 20% of the brewed extract) such as soluble sugars and different syrups.

One other measure of the wide variety of different beers and brewing techniques can be seen from the amount and types of different malts. Pale ale and bitter are made from, mostly, ***pale malt*** which is dried at temperatures low enough to retain the activity of the grain enzymes (i.e. the malt has a high diastatic power, DP). The color is therefore light and is often used as base malt (the major component in the grist) in many beers. Pale lager is made using ***pilsner malt*** (lager malt) as the base wort component. The required enzymes are, also here, retained in the grains. Mild ale is made from ***mild malt*** which is kilned at somewhat higher temperatures than pale malt. This gives the beer a less neutral flavor usually called “nutty”. ***Amber malt*** is kilned at 150–160 °C and retains, therefore, no DP. This malt gives the brew an intense bitter flavor which matures with time and is used for brewing brown porter and many British beers. Stout beer main ingredient is ***stout malt*** which has a light color and a maximum DP. The high DP is needed to solubilize starch and proteins in dark malts and un-malted grains which are used in brewing stout beer. [1, pp. 27-67]

### 2.2 The brewing process

The brewing process starts with preparing the wort (**Fig. 1**). The malt (and possible adjuncts) is **mashed** with the brewing water [1, pp. 101-186]. The sugars and amino acids, liberated during malting, are transferred into the hot liquid. Some hydrolyzing enzymes might still be active in the malt, depending on the used temperature during malting. This resting enzyme activity (enzymatic rest e.g. β-glucanase-, protease-, and amylase rest) can be utilized during mashing, to liquefy carbohydrates and proteins. Mashing temperature should thus be raised slowly according to the optimal temperatures of the mashing enzymes; 40–45 °C for β-glucanase, 50–54 °C for protease, 62–67 °C for β-amylase, and 71–72 °C for α-amylase. The slurry is then filtered (lautered) and the resulting **wort**, is mixed with selected **hops** (and other possible ingredients) and boiled in the brewing kettle. The function of the hops, besides adding bitterness and flavors to the beer, is to hinder the growth of micro-organisms in the wort after sterilization during boiling. Possible solids are removed, after boiling, and the mixture (the hopped wort) is transferred to a fermentation tank. The slurry is cooled down until a temperature is reached where the yeast can grow and work safely (**Fig. 1**). [1, pp. 17-23]

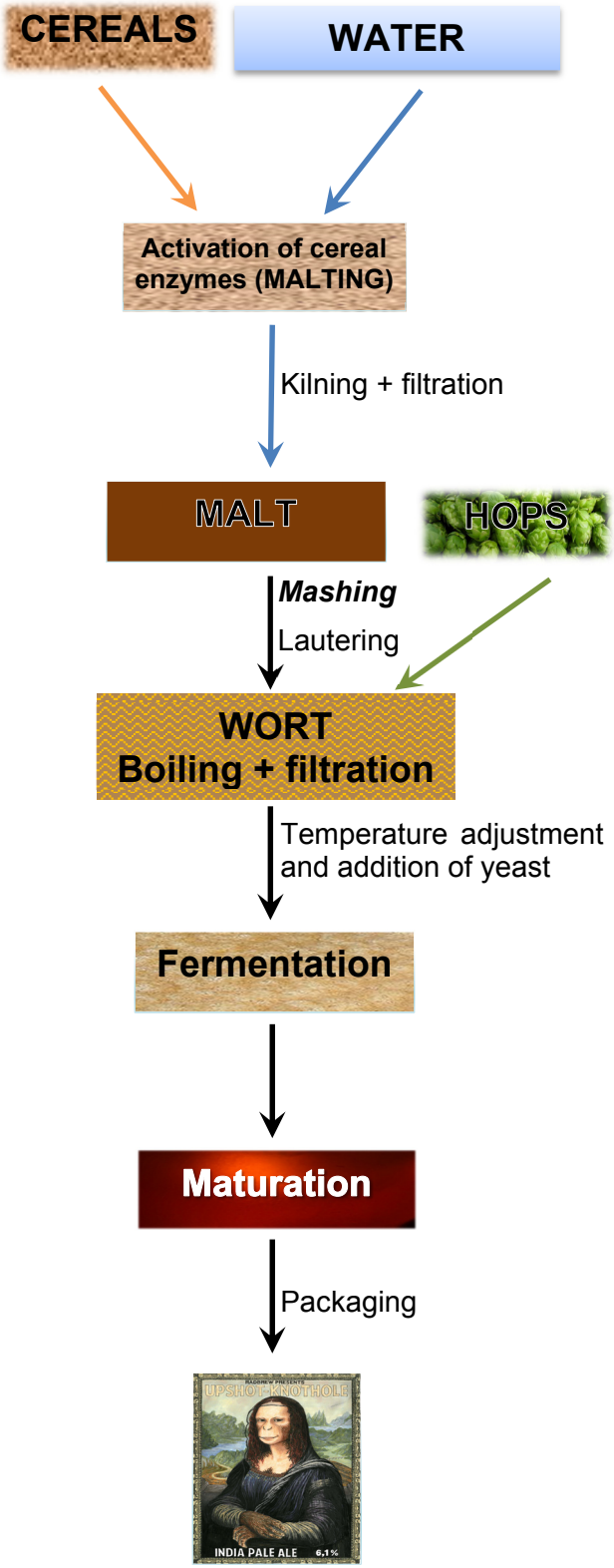



**Figure 1. The brewing process outlined.**
A schematic overview of the brewing process, including production of the malt.

The fermentation, either bottom- or top fermentation, is performed at strictly regulated conditions until the desired concentration of alcohol is reached. The fermentation starts by ***pitching*** the yeast which means, simply, that the yeast is added to the wort and the fermentation is thus initiated. Before the yeast is added the wort has to be cooled (to below 27ºC) and aerated properly. The specific gravity is measured before the yeast is added (suspended into a small amount of wort) to the main batch. [1, pp. 379-558]

### 2.3 Basic types of brewing techniques and types of beers

Below is a short list of some basic beer types. Most beers are made using some combinations of ingredients used for ales, lagers, pilsner and stouts/porters.

An ***ale*** has a sweet-, fruity-, and full-bodied flavor and is produced using a so called warm brewing method and the yeast species *Saccharomyces cerevisiae*. The temperature is usually ~15 – 24 ◌C and a foam is formed on the top of the ferment (also called "top-fermenting" or "top-cropping"). The bittering agents were earlier herbs and spices but today they have been replaced with hops. There are several types of ales with distinct characteristics much depending on hop amount and quality, e.g. *pale ale* (bitter), *India Pale Ale* (IPA), *brown ale*, *Scotch ale*, *American Pale Ale* (APA), *Burton ale*, *mild ale*, *Belgian ales*, *golden ale*, old ale, and *barley wine*. A ***pale ale (or "bitter")*** is made using mostly pale malt and abundantly hops, giving it a light color and often a hoppy flavor.

***Lager*** (from the German word "*lagern"* for "storing") style beers are pale lagers matured and conditioned at low temperatures. Lager beer is also produced using a specific, bottom-fermenting, yeast, *Saccharomyces pastorianus*, which is different from the ones used for brewing ale. Strong lagers (alc. >5,8 vol.%), of German style, are called **bock** and they can be dark, amber or pale in color. They can thus be of many styles varying in hop content, color and alcoholic strength (e.g. helles bock, doppelbock, eisbock and weizenbock)

A ***pilsner*** is a pale lager-type beer with a distinct hop aroma and flavor. The pilsner beer styles can be grouped into Czech-, German-, and European-style pilsner and has usually an alcohol content of 4,5 – 5% (vol.%).

***Stouts and porters*** (e.g. dry stout, Baltic porter, milk stout, oyster stout, imperial stout) are strong (7 – 8 vol.%), beers, made using roasted malt (or barley), hops, water and yeast. Porters and stouts are in many cases synonymous and, thus, today stouts are associated with very dark colored beers. Historically stout and porter meant the same

brew although the denotation “stout” was often used for strong porter. Today porters are characterized by a flavor of roasted malted barley while stouts are brews earlier denoted as dry stouts. The malt used for stout contains usually a small amount of un-malted roast barley and sweet stouts, for example milk stout, are also seen as typical stouts.

Other types of beers are for example ***weizenbier*** (beer made using predominantly malted wheat), ***lambic*** (a Belgian beer which is fermented using wild yeasts or Lactobascillus bacteria). [1, pp. 17-23]

### 2.4 Main flavor compounds in hops and malt

Hops are the flowers of *Humulus lupulus* and, originally (in the middle ages), the hops were included in the brewing of beer predominantly because of its ability to prolong the shelf-life of the beer. Other herbs were also used but the best suited additive seems to have been hops because of its pronounced bacteriostatic properties and the bitter and hoppy flavors it added to the otherwise sweet brew. Hops account for most of the flavor components in beer. It is therefore crucial that the selected hops are of an excellent quality. The flavor components in hops are very complex mixtures of volatile and semi-volatile compounds. Recommendations by the Nomenclature Committee of the Hops Liaison Committee defined ***non-specific fractions*** and ***specific compounds and mixtures of specific compounds*** [1, pp. 271-321].

*The non-specific fractions* are:

Those hop constituents that solubilize in both cold methanol and diethyl ether (excluding hop wax) are called **total resins**. These compounds include hard resins, uncharacterized soft resins and $\alpha$- and $\beta$-acids.

The **total soft resins** are those that solubilize in hexane (principally $\alpha$- and $\beta$-acids and uncharacterized soft resins).

The **hard resins** are those compounds, in the total resins that do not solubilize in hexane, and they are thus calculated by subtracting total soft resins from total resins.

A subtraction of the amount of $\alpha$-acids, from the total soft resins. gives the **$\beta$-fraction**.

The uncharacterized compounds, included in the total soft resins, are called **uncharacterized soft resins**.

*Specific compounds and mixtures of specific compounds* are:

The **$\alpha$- and $\beta$-acids** which are mainly, humulone, cohumulone, adhumulone, and lupulone, colupulone, and adlupulone, respectively.

The **α- and β-acids analogues** isohumulone, isocohumulone and iso-adhumulone (all mixtures of cis- and trans-isomers).
The **allo-iso-α-acids** (α-acid isomers, both cis- and trans) e.g. cis- and trans-allo-isohumulone, cis- and trans-allo-isocohumulone, cis- and trans-allo-isoadhumulone.
The **hulupones** (hulupone, cohulupone and adhulupone.) which are oxidation products of β-acids.
The **humulinic acids** (both cis- and trans forms) cohumulinic- and adhumulinic acid.

The α- and β-acids are the most important components for preventing microbial growth. Many of the compounds undergo transformations during wort boiling, e.g. oxidation and isomerization. It is therefore common that brewers add more valuable hops (aroma hops) to the wort at the end of the boiling so as to preserve desired, original, hop flavors in the brew. This procedure is called ***late hopping*** which must be distinguished from ***dry hopping*** where selected hops are added to the beer just before bottling [1, pp. 243-270, 2]. Besides organic acids and polyphenols, hops contain important compounds, for the beer taste and mouthfeel like terpenes, resins, essential oils and esters.

### 2.4.1 Alpha- and beta-acids

The α- and β-acids (humulones and lupulones, **Figs. 2 a and b**, respectively) are oily substances from the hops (the lupulin glands) that add bitterness to the beer (after transformation reactions) and possess antiseptic properties, in inhibiting efficiently the growth of Gram positive bacteria. There are several analogues of both α- and β-acids differing in the composition of the acyl side chain. The antiseptic activity is attributed to the prenyl groups which cause leakage of the bacterial primary membrane [3]. Both the α- and β-acids undergo transformations during wort boiling which enable them to solubilize into the wort. The transformations are mainly isomerization and oxidation reactions which can also be detrimental to the flavor. The most unfavorable reactions are those, induced by light, that occur between iso-α-acids and riboflavin (from the malt) producing compounds with an unpleasant taste

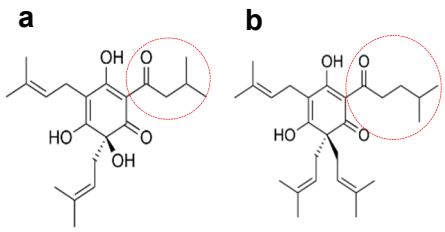



**Figure 2 a and b. Chemical structures of humulone and lupulone.**

The α- and β-acids humulone (a) and lupulone (b), respectively. The red dotted circles show the placement of the acyl chains that give rise to α- and β-acid analogues.
Structures modified from the Wikipedia (https://en.wikipedia.org/wiki/)

("lightstruck" beer). The reactions involve the formation of free radicals, by homolytic cleavage of exocyclic carbon – carbon bonds.

Isomerization of α-acids into iso-α-acids takes place during wort boiling and proceeds by conversion of humulone to isohumulone (cis- or trans-iso-α-humulone), and further to humulinic acid (**Figure 3**). The latter is found in only trace amounts in beer, when it is brewed using conventional methods. Practically all α-acids undergo isomerization so that only trace amounts of them are left in the mature beer. The humulone and the humulinic acid do not have bitter flavors. Apart from humulone other α-acid analogues such as adhumulone, cohumulone, posthumulone, and prehumulone are commonly found in hops. The major formed bittering agents, in α-acid isomerizations, are the iso-α-acids isohumulone, isocohumulone, and isoadhumulone.


**Figure 3. Isomerization of humulone**

The major isomerization pathway of humulone to humulinic acid, via iso-α-humulone. The production rate of humulinic- and iso-hexenoic acid is slow and the concentrations, in beer, of these compounds are therefore small.

Also the β-acids are found as analogues and the most frequently occurring are called lupulone, colupulone and adlupulone. The β-acids dissolve poorly into water and contribute therefore only little to the beer flavor. They are, however easily degraded, by oxidation, into compounds that have an unpleasant taste. The β-acids are usually unwanted


**Figure 4. Isomerization of lupulone**

Oxidation pathway of the β-acid lupulone.

because of their tendency to oxidize although the bitter hulupone contribute to the beer flavor.

The α- and β-acids are very insoluble in water, compared to their iso-acids which are readily soluble and bitter.

[1, pp. 271-321]

### 2.4.2 Essential oils, terpenes, and terpenoids

***Essential oils*** are constituted largely of terpenes and terpenoids. The essential oils are found in many plants and they are often wanted for their fragrances (perfumes), for the use in alternative medicine (e.g. aroma therapy), and for giving flavors to foodstuffs (beer). A large part of the aroma and flavor in beer is derived from the essential oils. The oils are usually very volatile and up to 250 of these substances have been identified in hop essential oils so that all hop varieties have their own characteristic oil composition

pattern [4]. Plants, especially conifers, produce hydrocarbons called ***terpenes*** (derived from the word turpentine) that protect the plants by deterring herbivores and/or by attracting parasites that feed on the herbivores. Terpenes serve also as central biosynthetic building blocks in almost all living organisms. The terpenes are sticky substances with a strong odor and they are the main constituents in resin. The ***terpenoids*** (also called isoprenoids) are terpenes with additional functional groups. The principal unit in terpenes is the isoprene ($C_5H_8$, **Figure 5**) unit which can be linked together forming linear-, or ring formed molecules. There are, thus, a large variation in the terpene types so that the prefix in the name indicates the number of isoprene units, e.g. hemiterpenes (only one isoprene unit), monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), diterpenes (four isoprene units), sesterterpenes (five isoprene units) and triterpenes (six isoprene units), etc. The terpenes in hops are principally (~90%) monoterpenes (myrcene, linalool, limonene) and sesquiterpenes (humulene, farnesene, and caryophyllene) so that ~80-90% of them are humulene, caryophyllene, and humulene. Terpenes add flavors like citrus (myrcene), orange (limonene), spicy aroma (caryophyllene), pine (pinene, myrcene, and limonene), herbs (myrcene), floral (geraniol) tropical fruit, etc., to beer.

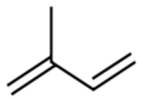


**Figure 5. Structure of isoprene**
The chemical structure of the isoprene unit

The volatile nature of the terpenes (and $\alpha$-acids) cause substantial losses (usually 50 – 90%) of the original volatiles amounts, during brewing. To overcome this problem many brewers have developed techniques how to add the desired flavors into the brew, later in the brewing process. By late hopping a brewer can add a part of the total hops (or specially cultivated aroma hops) to the wort near the end (e.g. during the last 10 minutes) of boiling. Another much used technique is to add aroma hops to the brew after wort cooling (before or during fermentation) by dry hopping. Because of the reactivity of the terpenes (with e.g. Sulphur containing compounds), the hopping method (late-, or dry hopping) matters in that different aromas are formed [1, pp. 271-321].

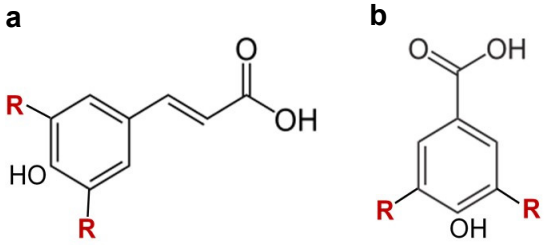
### 2.4.3 Esters and aldehydes

Esters are formed in reactions between organic acids (from hops) and alcohols (produced during fermentation) during the later parts of brewing. The formed esters can add crucial (fruity) flavors to, especially, ale-type beers. Aromas given by esters like isoamyl

acetate (banana), ethyl acetate (pear), ethyl hexanoate (apple/anise), and ethyl butanoate (tropical / pineapple) are often found in beers. Acetaldehyde will give apple flavors to the beer.

### 2.4.4 Phenols and polyphenols

Both malt and hops contain phenols and polyphenols that are important for the flavor and mouthfeel of beer. Hop polyphenols are mainly phenolic acids such as hydroxybenzoic acids and hydroxycinnamic acids. Hops contain also flavonols (mostly as glycosides). Hop polyphenols are found as monomers, dimers, trimers and complexes with nitrous compounds. Although hop polyphenols are important the majority (70-80%) of polyphenols in beer comes from the malt [2, 5]. Barley contain many phenols e.g. tyrosine, tyramine and hordenine. Barley contain, additionally, several phenolic acids which can be divided into two main groups (**Figures 6 a and b**). The substituted cinnamic acids (most abundant in malt is ferulic acid) and the substituted benzoic acids (most abundant in wort is vanillic acid) which can be found both free and in combination (as esters) with e.g. carbohydrates like glycosides and sugars. [1 pp. 101-186]




**Figures 6 a and b. Chemical structure of phenols.**

The chemical structures of the phenolic acid main groups, cinnamic- (a) and benzoic acids (b), respectively. The points of substitution are marked with a red R.
Basic structures modified from: NEUROtiker - Own work, Public Domain, https://commons.wikimedia.org/w/inde.g.php?curid=1378738 and - curid=3228267

### 2.5 Important sampling points for beer quality analysis

The selection of the time-point during the brewing process (sampling moment), and compounds to be analyzed, can be difficult especially if the objectives of the analysis are vague. Proper product control obviously demands that at least the end product (the beer) is analyzed and assessed. However, several sampling points can be selected if the intention is to monitor the whole brewing process.

### 2.5.1 Raw materials

The most important beer components are the water, the starch source, and the hops. The main component in beer is water which must be clean and available in large quantities. Water quality affects directly on the beer quality so that if inadequate quality water,

or contaminated with even a single solubilized foreign element, the beer value might be affected negatively. Naturally occurring calcium minerals, such as calcium- bi-carbonate, or sulphate, will have a positive impact on the beer taste provided that the kind and nature of these minerals are considered when choosing the type of beer to be brewed. Generally, hard waters (containing much minerals) are better suited for stouts while soft waters (containing less minerals) are more appropriate for pale lagers. An example of manipulation of water quality, to favor flavor release from the hops, is Burtonisation (from Burton upon Trent, a town in England) where gypsum (sulphates) is added to the water in order to brew good quality pale ales. Another way to manipulate water is to add vitamins and minerals to the water to produce Irish stout-like beers (Guinness).

The quality of the starch source, usually barley, is crucial for the produced beer. Barley is cultivated in many varieties and the grains differ with respect to size and constituent qualities [1, pp. 27-30].

### 2.5.2 The malting process

The plant (seed) growth commences during malting which means that the synthesis of several hydrolytic enzymes starts. Besides starch hydrolyzing enzymes, also proteins, hormones and lipids begin to metabolize. The most important compounds, for the beer taste and properties, are the nitrogenous (proteins, amino acids and melanoidins) compounds released during malting. There are several similar analysis methods (e.g. nitrogen/protein determinations) in use because of many differing malting methods and brewing traditions. These compounds are very important, not only for the beer flavors and aromas, but also for the beer color and foam quality. Malt analysis show that typical ranges of e.g. non-protein extract, crude protein, ash, and fiber, to be 35-50%, 20-35%, 6-8%, and 9-15%, respectively. However, the methods differ significantly depending on brewing technique and which recommendations the brewery follows (IoB, EBC, ASBC, or MEBAK). The nitrogen (protein) analysis is the most important of the analyses when considering flavor and quality (e.g. beer foam and mouthfeel). Usually, total soluble nitrogen (TSN) and free amino nitrogen (FAN, mostly small peptides and amino acids) are measured but often the SNR (Soluble Nitrogen Ratio = TSN / total nitrogen, IoB method) is given for the malt. The SNR can also be given as Kolbach Index (soluble protein / total protein ratio, ASBC and EBC methods). Other important measurements are % humidity and diastatic power (hydrolytic enzyme activity). Brown compounds called melanoidins, formed through Maillard reactions between reducing high molecular weight sugars (large

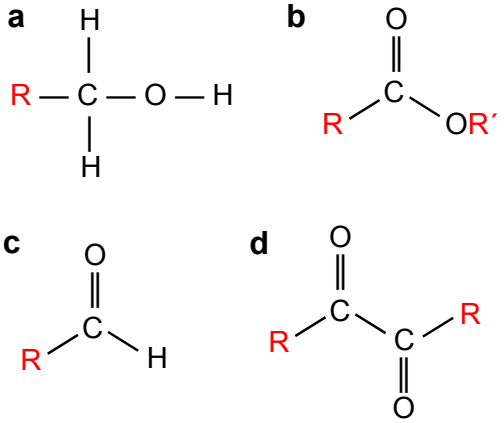
polymeric dextrins) and amino acids, are determined by measuring the color intensity. Contaminant analysis, e.g. iron, cadmium, arsenic and lead, must occasionally be measured as well as determinations on the levels of microbes (esp. *Fusaria*), pesticides, and halogenated contaminants [1 pp. 27-67]. The malting process is thus important seen from the quality control point of view. However, the emphasis of analysis is much on the used raw materials and the end product rather than on the process.

### 2.5.3 The wort

Most compounds important for beer taste, are added to the brew during wort boiling (see **2.4**). In this process, the resins (including humulones and lupulones), polyphenols, esters, aldehydes etc., are solubilized into the wort which makes the wort-making process an obvious point of sampling.

### 2.5.4 During fermentation

Fermentation is a step in brewing, where drastic changes in the brew composition takes place. Therefore, the analysis of the fermenting wort can be performed from several viewpoints. One point of view is to follow the development of components that are important for the final flavor of the beer. Another need for analysis could be to check the efficiency of the process, and a third to monitor off-flavors caused by other micro-organisms such as bacteria and wild yeast. The most important beer flavor affecting yeast metabolites are ethanol and higher alcohols (**Figure 7 a**), and carbonyls like esters, aldehydes and vicinal di-ketones (**Figures 7 b-d**). Yeast excretes also organic acids like lactic-, citric-, pyruvic-, malic-, acetic-, formic-, succinic- and butyric acid into the medium, which also affect the taste of the final product. The formation of carbon dioxide ($CO_2$) is crucial, especially for the mouthfeel of beer and to maintain anaerobic conditions.




**Figures 7 a-d. Structural formulas**

The structural formulas of **a**) methanol and higher alcohols, **b**) a carboxylate ester or a carboxylic acid if the R´ is a hydrogen atom, **c**) aldehydes, **d**) a vicinal di-ketone (VDK). R and R′ denote any alkyl or aryl group. R and R´ can also be a hydrogen atom.

The mostly used species of yeast, for brewing, are ***Saccharomyces cerevisiae*** and ***Saccharomyces pastorianus*** (formerly "*S. Carlsbergensis*"). *S. cerevisiae*, also called

“brewer’s yeast”, is also well known to bakers and wine makers although the strains are different. *S. cerevisiae* is a top fermenting yeast, forming a foam at the surface of the fermenting wort, while *S. pastorianus* (called “ale yeast”) is a bottom fermenting yeast and precipitates thus at the bottom of the brewing vessel. The yeasts are also called “top”- and “bottom cropping”, respectively.

The complex metabolic reactions (pathways), catalyzed by enzymes, can roughly be divided into **catabolic** and **anabolic**. During catabolic reactions, organic compounds like starch (and very large carbohydrate polymers), proteins and fatty acids are degraded into their constituents (glucose, amino acids and acyl-Coenzyme A, respectively) at the same time as energy is produced. The anabolic reactions consume energy while producing vital components for yeast growth and bi-products like ethanol, $CO_2$, and esters. These metabolic events (pathways) are highly regulated and it is thus, from the brewer’s point of view, essential to control the outcome of the processes. For example, the formation of higher alcohols (from aldehydes) and ketones (from amino acids) as well as the reduction of vicinal diketones (VDK, by-products in biosynthesis of the amino acids valine and leucine), are important beer flavor affecting reactions. Regulation occurs at both gene- and metabolic levels (protein synthesis and enzyme activity, respectively) so that the produced compounds might inhibit the formation of the same compound. Strict regulation is needed for the organism to maintain a balance between redox (reduction and oxidation) reactions. During aerobic growth (respiration), cell respiration is triggered to direct the energy production through the so-called electron transport chain where oxygen is the final electron acceptor, resulting in the formation of energy (ATP), water, and $CO_2$. The large number of ATP produced lead to a considerable increase in the yeast cell population. When the dissolved $O_2$ in the wort is depleted, anaerobic respiration takes place. Anaerobic respiration (**fermentation**) means that acetaldehyde is the final electron acceptor (instead of oxygen) producing, ultimately, energy (ATP) during the production of pyruvate and its conversion to acetaldehyde and then ethanol. Fermentation is thus an anaerobic process which is incomplete with respect to oxidation of organic compounds. Only 2 ATP are produced from glucose during fermentation, so yeast metabolism speeds up nearly 10-fold, rapidly consuming glucose, in order to produce sufficient energy for the yeast cell. The fermentation of one molecule of glucose yields two molecules of ethanol and carbon dioxide according to the formula: $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$. Fermentation is an exergonic (exotherm) reaction, where produced energy is released as heat. The yeast metabolism is very sensitive to disturbances, and thus, to beer quality. The biochemical reactions during fermentation, deplete the wort of nutrients

while ethanol, carbon dioxide and metabolites are formed. Important cellular mechanisms, like uptake of nutrients and the transport of nutrients and metabolites, between cellular compartments, are strictly regulated and can thus be “bottlenecks” in yeast function if the conditions are not optimal. It is, for example, common in biochemical reactions that metabolites inhibit the formation of one or several products or cellular mechanisms. The level of different compounds can serve as markers on the state of the process so that, for e.g., a high concentration of **glycerol** is a sign of stress, caused by a high osmotic pressure (a high concentration of solutes). Another important marker is the pH of the fermenting wort. The yeast needs nitrogen for growth but it is not able to utilize (assimilate) atmospheric nitrogen. The most important nitrogen sources are amino acids, peptides, amines, purines and pyrimidines, primarily originating from the malt.

Minerals, especially sulphur (from sulphur containing amino acids) and essential minerals for catalytic- and other biochemical reactions, like $B^{+}$, $Ca^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Fe^{3+}$, $K^{+}$, $Mo^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Ni^{2+}$ and $Zn^{2+}$, are needed, in adequate amounts (<10 µM) for proper function of the yeast. Organic growth factors like vitamins, certain lipids, nucleosides, nucleotides and some purines and pyrimidines are obligatory for the yeast metabolism and growth. Some yeast strains also require essential compounds like biotin, inositol, thiamine and pantothenic acid.

A very large number of different metabolites are formed during fermentation or as a result of shock excretion (caused by, for example, a high concentration of a certain metabolite or unfavorable temperatures or pH) or cell death. However, the concentration of many of the metabolites is so small that, at normal conditions, the flavor is not affected. Organic- and fatty acids, aliphatic (non-aromatic) alcohols, and esters of alcohols, are the most important metabolites affecting beer flavor. These compounds are thus the main objectives of analysis.

The most important compounds of interest are:

***Alcohols (Figure 7 a)***

***Organic-*** (acetic-, citric-, lactic-, malic-, α-ketoglutaric-, pyruvic-, and succinic-), ***and fatty acids*** (short- and medium chain length fatty acids, $C_6$ – $C_{18}$), are off-flavors and inhibit beer foam formation.

***Carbonyl compounds*** contain C=O functional groups (**Figures 7 b-d**), such as aldehydes, carboxylic acids, and vicinal di-ketones, are common in beer. Many are, however, detrimental to the beer taste and therefore the concentrations of these compounds should be minimized. Acetaldehyde, which is the precursor for ethanol, is an important

compound. Acetaldehyde might accumulate to an excess during fermentation, and it will give the brew an unpleasant flavor and aroma if the flavor threshold (10 – 20 ppm,) is exceeded. The most common reasons, for too much acetaldehyde in the brew are the use of a poor-quality yeast, a too high temperature during fermentation, a too fast addition of the yeast or a too high pitching rate, and a too high level of wort oxygenation [1, page 472]. Other important carbonyl compounds, especially for lager beers, are VDK: s, diacetyl (2, 3-butanedione) and 2, 3-pentanedione. These compounds are harmful for the beer taste if their flavor thresholds are exceeded. VDK: s are by-products that form during the synthesis of the amino acids valine and isoleucine and their formation is connected to the FAN concentration (the selection of produced amino acids) through metabolic biochemical processes [1 pp. 417-485].

### 2.5.5 The beer maturation process

Beer components constantly undergo equilibrium reactions where the concentration of some flavor components diminish and others increase. Some components can even disappear or an entirely new species can form. Beer flavor deterioration reactions are thus a result of both synthesis- and degradation reactions, which should be considered already when the raw materials for the beer are chosen and their weights are calculated. Aging flavors and colors are therefore not usually regarded as off-flavors, except if the consumer is accustomed to a special brand with a specific taste [6]. Aging reactions will ultimately reach a state of chemical equilibrium where the reactions proceed only slowly. The flavor components in mature beer will have a distinct (analysis) profile showing how well the flavors correspond to the planned taste. The content of e.g. $\alpha$- and $\beta$-acids have an impact on the bitterness and the amounts of terpenes will affect the fruity and spicy flavors of the final product. Bitterness (the content of, especially, $\alpha$-acids), fruity (estery) and floral aromas tend to decrease, while the development of sweet aroma components will increase, during aging. A strict distinction, between beer- staling and aging, is sometimes difficult to make since same components affect both phenomena [7]. The emergence of typical aging flavors during beer storage can be linked to Maillard reactions, the formation of linear aldehydes, ester- formations and degradations, acetal formation and etherification and degradation of bitter compounds from hops. Carbonyl compounds, especially unsaturated long chain aldehydes having a low taste and odor threshold, are readily formed during beer storage [6,8,9]. Beer aging, as a phenomenon, is thus a very complex issue which has been the subject of much research [10]. Many of the introduced flavors (mainly from the hops) undergo changes during the beer maturation process. The

changes are of chemical nature and are various forms of breakdown reactions, for example de-carboxylations, isomerizations and Maillard reactions. Especially compounds important for beer flavor (volatiles) will undergo Maillard reactions and can be used as markers for beer aging [11]. These types of reactions are common among, especially, the polyphenols, flavonoids and diketones. One important group of compounds are the vicinal diketones (VDK) which are often used as markers of the beer maturity. The VDKs give beer a buttery flavor and are therefore mostly unwanted. The beer is thus considered mature when the VDK levels have fallen under a certain level.

Beer contains several, both volatile and non-volatile, **sulphur containing constituents.** Often these compounds have a low flavor threshold and might therefore become a problem as many of them are off-flavors. A majority of the non-volatile organic sulphur compounds are proteins and peptides containing the amino acids methionine and cysteine found predominantly in the wort but can also be present in the final beer. Sulphur containing compounds are also found, in small amounts, in hop oil as polysulphides. Polysulphides are readily formed, especially if the hop oils are produced using steam distillation because of reactions between elementary sulphur and sesquiterpenes. Volatile sulphur compounds are preferentially analyzed using GC, equipped with a headspace sampling apparatus, because of their reactivity in conditions where they are exposed to light or oxygen. The perhaps most important volatile sulphur containing compound is dimethyl sulphide (DMS), which is an off-flavor, typically found in lager beer [1 pp. 707–710].

### 2.5.6 Beer staling

The chemical processes taking place and tastes emerging during beer- aging and staling are much the same. However, beer aging differs from staling in that aging is usually seen as a positive phenomenon, where a (planned) flavor balance is reached between the flavor components, while staling is associated with beer spoiling agents like microorganisms, $O_2$, too elevated temperatures, and unwanted chemical reactions induced by light, etc. Beer staling is not only noticed by the appearance of off-flavors but it is also characterized by the disappearance of the pleasant fresh flavors. Different types of beers have their own distinct stale off-flavors which, when found in another beer type, might not be a problem (when present in reasonable concentrations). The stale and aging flavors are often difficult to pinpoint by tasting because other beer components might mask their taste or the off-flavor concentrations can be just below their flavor olfactory thresholds [7,11]. The flavor of staling is often referred to that of cardboard (especially for lager-type beers), sweet-, caramel- or toffee-like-, “ribes-”, and burnt-sugar flavors. The source for

these tastes is the perhaps predominant off-flavor compound occurring during staling, an unsaturated aldehyde ***(E)-2-nonenal***. This compound occurs especially if beer has been stored in elevated temperatures. Much research has been done to elucidate the primary source of (E)-2-nonenal and it seems most likely that it is formed by oxidation of lipids (mainly the unsaturated fatty acids, linoleic-, and linolenic acid) during mashing or wort boiling [10]. Mashing activates several fatty acid degrading enzymes which produce (by oxidation) precursors to carbonyl compounds like (E)-2-nonenal.

Even the lowest contents of oxygen in the final beer will cause a rapid deterioration of flavor agents. Brewers try therefore to prevent oxidation reactions by deterring oxygen at all stages after malting and, especially, fermentation. Off-flavor agents, like ***Strecker aldehydes*** (e.g. 2-methyl propanal, 2-methyl butanal, 3-methyl butanal, benzaldehyde, phenylacetaldehyde and methional), are reported to increase at elevated oxygen concentrations. The origin of these carbonyl compounds is still under much debate and there are principally two main theories on the origin of these off-flavors. One model states that the compounds are released from a bound state during beer aging. Others have suggested pathways for their formation during wort production or in the final beer by Maillard reactions from oligosaccharides or by the influence of reactive oxygen species (e.g. $O_2^{\bullet-}$, $OH^{\bullet-}$, $HO_2^{\bullet-}$, and $H_2O_2$) and/or $Fe^{2+}$ through Fenton- or Haber Weiss reactions. Strecker aldehydes are, most probably, a result of the so called *Strecker degradation* of amino acids (mostly valine and leucine) under the influence of Cu- and Fe ions [6,10,12].

The temperature has a crucial importance in the development of staling reactions. Cool temperatures slow down the reactions while even a slight increase in the temperature above 4ºC will markedly increase the rate of beer staling reactions. Elevated temperatures can be used in quality control to predict the shelf life of the beer [13,14].

Beer is said to be “lightstruck” when a skunky off-flavor appears. These flavors are caused by sunlight, which induces photo-oxidation of hop derived compounds, e.g. isohumulones to MBT (3-methylbut-2-ene-1-thiol) via photosensitized riboflavin [15].

There are several other types of reactions, e.g. acetalization of aldehydes, Maillard reactions, synthesis and hydrolysis of volatile esters, degradation of polyphenols, aldol condensations, etc., affecting the quality and flavor of beer [1 pp. 678-731,10]. These reactions complicate further the analysis (and especially the interpretations of analysis results) of beer and sets an intense pressure on both the laboratory personnel and equipment, and on training, again accentuating the importance of careful planning when a laboratory is started.

# 3 ANALYSIS METHODS FOR BEER COMPONENTS

When choosing the appropriate analysis methods, for any purpose, the precise requirements must be recognized and followed. There are several organizations with their own recommendations for analyzes, e.g. IoB (The Institute of Brewing), EBC (European Brewery Convention), ASBC (The American Society of Brewing Chemists), and MEBAK (Brautechnische Analysenmethoden: Methodensammlung der Mitteleuropäischen-Brautechnischer Analysenkommission). Proper analysis method choices are especially important when analyzing foodstuffs, already because of the strict regulations imposed by the legislation. The following requirements were taken from the book Analytical Chemistry of Foods [16] and show clearly the types of demands that one should set on the chosen analyzes:

**Precision** is a measure of the ability to reproduce an answer between determinations performed by the same scientist or by different scientists in the same laboratory using the same procedure and instrument.

**Reproducibility** is similar to precision, but based on the ability to reproduce an answer by different analysts and /or laboratories using the same procedure.

**Accuracy**, expressed in terms of the ability to measure what is intended to be measured; e.g. the fat content of a foodstuff rather than all substances of similar solubility's, or the protein content of a food rather than all nitrogen containing substances.

**Simplicity of operation** is a measure of the ease with which the analysis may be carried out by relatively unskilled workers.

**Economy** expressed in terms of the costs involved in the analyses in terms of, reagents, instrumentation, and time.

**Speed,** based on the time to complete a particular analysis. Important in cases where follow-up actions need to be undertaken quickly. For example, if some (unwanted) component(s) appear in excess or are too low.

**Sensitivity** measured in terms of the capacity of the method to detect and quantify food constituents and / or contaminants at very low concentrations such as might occur with trace elements or pesticide residues.

**Specificity** expressed in terms of the ability to detect and quantify specific food constituents even in the presence of similar compounds, e.g. individual sugars (e.g. reducing and non-reducing sugars).

**Safety**, many reagents used in food analysis are potentially hazardous, e.g. corrosive acids flammable solvents.

**Official approval** is crucial because various international bodies give official approval to methods that have been comprehensively studied by independent analysis and shown to be acceptable to the various organizations involved. E.g. ISO (International Organization for Standardization), AOAC (Association of Official Analytical Chemists).

Beer is a complex mixture of components containing more than 450 characterized substances. Beer contains chemical compounds such as proteins, nucleic acids, polysaccharides, and lipids. Most identified components affecting beer flavor are smaller compounds like organic- and amino acids, aldehydes, ketones, esters, alcohols, essential oils, resins (mostly terpenes and terpene alcohols), polyphenols (e.g. flavonoids), lipids and sulphur containing compounds. For convenience, the solubilized components are often divided into volatile and non-volatile substances where the former have grater vapor pressure and give beer its aroma (bouquet). The latter substances (such as, sugars, amino acids, polyphenols, inorganic salts, nucleotides, and hop resins) remain solubilized in the beer contributing to the flavor and mouthfeel of the beer. There are, thus, a myriad of possible components to analyze. Volatile compounds are concentrated in the headspace and can conveniently be analyzed using gas chromatography with mass-spectrometric detection (GC-MS). The non-volatile constituents include also macromolecules such as proteins, nucleic acids, and polysaccharides. These compounds are usually analyzed using high pressure liquid chromatography (HPLC) [1, pp. 678-731].

### 3.1 Traditional analysis methods

Traditional analysis methods are often time consuming and even vague for today's demands for production speed and efficiency. Economic facts dictate that the brewing process must be planned carefully and monitored for faults and essential component levels (e.g. the ethanol content and organic acids). This means that a large number of samples have to be analyzed, in a relatively short time, for the process to run smoothly. Many older methods require laborious extraction steps where the material is treated with polar

and non-polar solvents and then the extracts are concentrated and analyzed using, usually, some chromatographic methods. Physical methods have also frequently been used, e.g. for measuring the ethanol content. These methods involve often the measurement of the relative density (or specific gravity), using instruments like a pycnometer, an oscillating U-tube, or a hydrometer. The old traditional methods (before the development of chemical analysis methods) rely often on the taste- and smell senses which, although being relatively fast and cheap, can produce errors depending on the person who performs them.

### 3.2 Modern analysis methods

The development of chemistry, and especially chemical separation methods, have enabled analysis of individual compounds in a relative short time. Especially the development of the chromatographic methods, where mixtures of compounds are analyzed, enables an exact quantification of even minute amounts of compounds. Chromatography is based on the separation of dissolved (in the *mobile phase*) molecules when they are passed through a chromatography, by means of a mobile phase, column holding a *stationary phase*. The separation, of the dissolved compounds, is based on differential partitioning between the mobile and the stationary phases so that molecule species travel through the column at a different speed. The retention of molecules to the stationary phase (the partition coefficient, **P**, of the compound) is thus the key to the separation of compounds in the chromatography column. Flavor components are usually analyzed using gas chromatography because these components are predominantly volatile. Liquid chromatography (HPLC) is also frequently used and the target analytes are then usually carbohydrates, hop acids, the essential oils, and hop flavonoids in ex the identification of hop varieties [17].

#### HPLC

HPLC, or High Pressure (sometimes Performance) Liquid Chromatography, is an analysis method by which chemical compounds can be separated from each other and thus identified and quantified. HPLC is a fast and therefore a frequently used analysis method of, practically, all types of especially organic molecules e.g. proteins, fats, alcohols, hormones, different toxins, drugs, amino acids, and so on. The sole requirement is that you must able to solubilize the analytes of interest into a liquid medium. The solvent can be aqueous (i.e. polar e.g. water or an alcohol) or an organic solvent such as acetonitrile (ACN), methanol, tetrahydrofuran (THF), or isopropanol. The acidity of the elution media

can be adjusted using various compounds such as tri-fluoroacetic acid (TFA), ammonium acetate or formic acid. A traditional HPLC column is relatively small, usually 10 – 30 cm long and having a diameter of 50 – 100 mm. The column is packed with small (~3 – 10 µm) particles (often silica beads) having polar surfaces (the stationary phase) and containing pores of varying sizes. The pores give the stationary phase a larger surface area as well as characteristics like optimal kinetics for e.g. bulky proteins (large pore size). Small column volumes and particle sizes demand a high and steady pressure to work. Pressures around 100 MPa are therefore not unusual in the HPLC which sets high demands on the hardware, especially on the pumps. The particle and the pore sizes play an important role in the separation efficiency of the HPLC system. Larger particles are usually used primarily for preparative separations where the analytical separation is not so important.

**Separation of analytes**

There are several types of HPLC to choose from when you decide on an HPLC analysis method.

***Partition chromatography*** is one of the classical chromatographic methods and separates the analytes based on their polarity. The partition chromatography uses an inert stationary phase onto (or into) which a polar solvent is retained. The analytes then diffuse into the polar stationary phase and elute depending on their polarity so that the more polar groups the analytes contain, the longer it takes for them to elute from the column. The time it takes for the analytes to pass through the column (the ***retention time***) is unique for each chemical compound, meaning that compounds can be identified by their retention time, provided that the conditions (e.g. temperature, pH, and ionic strength) remain the same. Polar solvents will thus shorten the elution time while hydrophobic solvents usually increase the retention time.

***Normal-phase*** chromatography separates analytes based on their affinity for a polar stationary phase. The polar interactions (e.g. hydrogen bonding and dipole-dipole interactions) between the stationary phase and the analytes are therefore crucial for the separation efficiency. The analytes interact with, and are retained by (adsorbed to), the stationary phase so that compounds having many polar groups (e.g. –OH, -$NH_3$ or phosphate groups) will be efficiently retained by the sorbent material in the column. The analytes can then be eluted from the column using a non-polar and non-aqueous eluent (mobile phase) such as chloroform. Thus, more polar solvents in the mobile phase will

shorten-, and non-polar solvents prolong the elution times. Also, the physical properties of the stationary phase play an important role in the separation process, especially if the packed particles contain pores. In these cases, the analyte size and bulkiness matter in that steric hindrance will affect the elution speed. The separation of structural isomers is therefore possible when these factors are utilized. The disadvantages with normal-phase chromatography is that the presence of protic compounds (chemical compounds containing labile $H^+$ groups like –OH and -$NH_2$ groups, e.g. in water, organic acids and different amines) will cause drifting of the base line and thus the reproducibility of retention times suffer.

***Reversed-phase HPLC*** (RP-HPLC) has therefore largely replaced both partition- and normal phase chromatography. In RP-HPLC the separation principle is “turned around” so that the stationary phase is non-polar and the mobile phase is aqueous or moderately polar. The non-polar (hydrophobic) analytes have thus affinity for the stationary phase while polar components elute from the column with the polar mobile phase. This experimental set-up increases the reproducibility of the experiments because the hydrophobic matrix is less prone to chemical reactions caused by the used eluents. Usually the stationary phase is composed of silica, coated with carbon chains being mostly 8 or 18 carbon atoms long (there are also 1-, 4-, and 12-carbon materials). These materials are called C8- or C18-bonded (octyl- and octadecyl carbon chain-bonded silica, respectively) silica. The difference (in separation efficiency) between these two coatings is not very big. The main difference between these two sorbents is in the degree of hydrophobicity which increases with a longer carbon chain. In general, on can say that a C8-coater material is better suited for the separation of smaller molecules e.g. organic acids while a C18 sorbent is more suited for the separation of e.g. long chain fatty acids. Generally, C8 sorbents result in shorter retention times and less selectivity. A too high polarity can result in so called “carryover” which is a situation where some analyte material will remain in the column (because of a too strong affinity for the sorbent material) and thus give rise to “ghost peaks” in subsequent runs. Columns available today can be of many types, having different polarities. The coating can, consequently, contain amino-, cyano-, pentafluorphenyl- or nitrile groups, according to the demand for stationary phase polarity. The analytes can be eluted ***isocratically***, meaning that the water-solvent contents are constant during elution, or using a two-component gradient (***linear gradients***) containing water (or aqueous buffers) and an organic solvent, that is miscible with water. Such organic solvents are acetonitrile, methanol, tetrahydrofuran, ethanol and 2-propanol. In

some cases, the mobile phase pH is crucial because the retention efficiency (selectivity of analytes, i.e. their polarity) might be dependent on the surrounding pH.

**Detection**

A HPLC system can have a wide range of detectors depending on the analytes and purpose of the analysis. Some of those are listed below.

The ***refractive index*** (RI) of molecules can be used to detect analytes after HPLC separation. The RI detector measures the refractive index of any dissolved compound (that differs from that of the solvent) and is thus capable of detecting the presence of all dissolved components. These detectors are called universal detectors because they indeed detect practically all molecules applied onto the column. However, the aims of most analyses are to detect and quantify only specific components and therefore we usually chose more specific detection methods.

An ***ultra-violet visible*** (UV-Vis) detector operates in the ultra violet- (200 – 400 nm) and the visible regions (400 – 700 nm), and sometimes even into the near-infrared (700 – 1400 nm) region. This is why this detection method is the most popular among the HPLC detection methods. In UV-Vis detection, the detector range is expressed in absorbance units (A). One absorbance unit corresponds to the depreciation of the light intensity by 90% of the incident light. Molar absorptivity, also called the molar extinction coefficient, corresponds to the absorbance for a molar concentration of the substance with a path length of 1 cm.

***Light scattering-*** (a less accurate detection method), ***fluorescence-***, ***flame ionization-*** (FID) and ***mass spectrophotometry*** (MS) ***detectors*** (very accurate detection methods) are other frequently used detection methods.

**Gas chromatography**

The chemistry in analyte separation using gas chromatography (gas-liquid partition chromatography, GLPC), is much the same as in liquid chromatography. The largest difference is that the *mobile phase* during GC is a gas which gives the analysis method many opportunities but also some challenges. The carrier gas must be inert to avoid reactions with the analytes. The most usual carrier gas is therefore helium but also nitrogen and hydrogen (unreactive) gases are used. The *stationary phase* is a very thin (microscopic) layer of an (inert) solid, liquid, or polymer, covering the inner side of a thin (glass or metal) tubing (column).

One obvious challenge is to solubilize (vaporize) the analytes without their decomposition, so that they can be carried through the capillary column by the mobile phase (gas). The introduction of the sample, into the gas chromatograph is done through the injector (also called the inlet) situated on the column head. There are several types of inlets that are suitable for different sample amounts and different physical states of the sample. Samples can be introduced into the GC as gases or liquids. Depending on the type of column inlet, the proper quantity of analytes is adjusted by fine-tuning the sample amount, or by regulating the volatilization temperature. In the analysis of beer aroma components, the sample is already in a liquid form and, thus, ready for analysis. Beer contains a lot of non-volatile compounds that will clog the GC and thus damage the capillary column. However, most of the beer analytes are volatile or semi-volatile making different head-space-, or SPME (Solid Phase Micro Extraction) techniques attractive for efficient analyte sampling [18,19,20,21,22]. In automated headspace techniques (e.g. dynamic- and static head-space technologies) the analytes are driven into the gas phase (called ***headspace***) by increasing the temperature, stirring, and /or by introducing a suitable amount of salt into the sample. The combination of heat, stirring, and salt addition are efficient means to vaporize when SPME is used for analysis of volatiles in beer [18-21]. The analytes are adsorbed onto thin fibers coated with adsorbing materials (coated fused silica) e.g. Polydimethylsiloxane (PDMS) which is a silicon-based organic polymer, Divinylbenzene (DVB), and porous carbon adsorbents (Carboxen® by Sigma-Aldrich). The analytes are then desorbed, by raising the temperature to ~250 – 290 ºC, and consequently transferred into the GC.

The separation of the analytes is accomplished by the differences in the analyte physicochemical properties and thereby due to their affinity to the stationary phase. The *retention time* will thus determine the identity of individual compounds.

**Sample treatment**

Beer and samples from different stages of brewing must be handled with care since many of the analytes are volatile and light and/or elevated temperatures might damage the flavor compounds. A GC, used for analysis of volatile components, is usually equipped with a headspace. The headspace technology samples gaseous components from the closed space where liquids or solids are placed. The principle of function is that an airtight seal is formed around the sample so that volatiles are concentrated in the void space. The volatiles are then transferred from the space into the GC column using vacuum and an inert gas. Another sampling technique, developed especially for volatile

compounds, is SPME (Solid Phase Micro Extraction) where a fiber, covered with a volatile collecting material (e.g. Carboxene, PDMS, DVB, or Polyacrylate) is inserted, using a specially developed syringe, into the headspace of a (closed and air tight) sampling vessel. Analytes are sorbed onto the collecting material and, once equilibrium is reached, the coated fiber is transferred to the injection port of e.g. a GC where the analytes (both volatiles and semi-volatiles) are desorbed from the fiber using heat (~230 - 290ºC). This technique has enabled rapid analysis of food flavor components and is therefore used increasingly in research [19,20,21,23]. The SPME technique allows also on-fiber derivatizations which increases the accuracy for e.g. carbonyl compound analysis [24]. SPME was especially developed for GC but this technique can also be used for HPLC analysis.

**Detection**

There are, as for HPLC, a wide range of detection methods for gas chromatography. The perhaps most frequently used analyte detection methods for GC analysis of beer aroma components are FID (Flame Ionization Detection) and MS (Mass Spectrometry).

The FID detector functions by detecting organic ions, produced by combustion of analytes in a hydrogen flame. Inorganic compounds and non-volatile compounds are thus not detected. The detection is accomplished by producing a potential difference using two electrodes (a positive- and a negative) placed before- and after (the positive- and the negative electrode, resp.) the flame. The ions induce a current by colliding (induced by attraction) with a collector plate. The formed current is then measured using a high-impedance pico ammeter and analyzed using a computer.

In mass spectrometry, ionized chemical species are produced and then sorted according to their mass-to-charge (***m/z***) ratio. The working principle of a mass spectrometer is relatively simple but different modifications and combinations with various ionization methods might complicate the general picture of the method. A simple description of the method is that the analytes are ionized and then directed through a magnetic field which will bend their path. The degree of deflection will depend on the analyte m/z-ratio so that heavier particles will deflect less than lighter particles. Generally, when MS is used in combination with GC, the used ionization methods produce single-charged ions (z=1), meaning thus that the detected signals can be regarded as the mass of the ion. Mass spectrometry is, simply, an instrumental technique where analytes are converted to positive ions by electron bombardment and then separated according to their masses (**Fig. 8**). A mass spectrum is, consequently, a plot of relative abundances versus the m/z.

Mass spectrometry is used when special accuracy is needed, for e.g. when researchers want to find and characterize unknown biological compounds. Beer is therefore an excellent object for studies because of its complexity and variability [1, 2, 5].

First, the sample analytes are separated into species in the GC. The species are then ionized by the ionizer and directed into a magnetic or electric field, where the ionized analytes are separated based on their mass to charge ratio (**Fig. 8**). The mechanism of ionization can be either by electron- (hard ionization) or by chemical (soft ionization) ionization. ***Hard ionization*** (performed using a large amount of energy) results often in the defragmentation of the analytes so that charged fragments, typical for the respective analyte species, are formed. ***Soft ionization*** means that analytes are ionized using a small amount of energy. GC-MS uses many of these ionization techniques, e.g. chemical ionization (CI), electron ionization (EI), positive- or negative chemical ionization (PCI and NCI, resp.), fast atom bombardment (FAB), electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). The subject molecules are, thus, fragmented only to a small degree and the resulting "base peak" (the most intensive signal) in the spectrogram is used for identifying compounds.

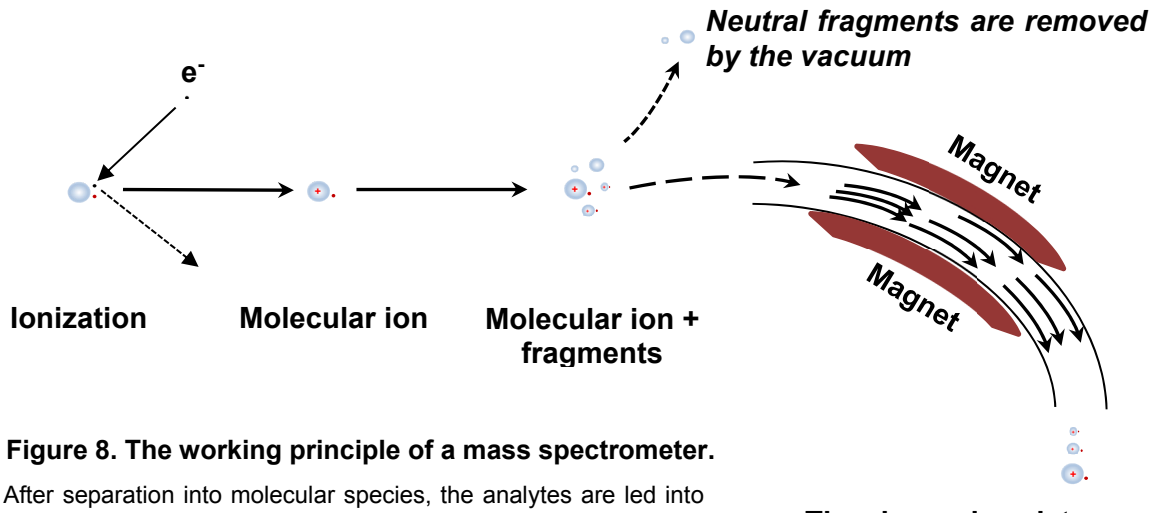



**Figure 8. The working principle of a mass spectrometer.**

After separation into molecular species, the analytes are led into the mass spectrometer where they are ionized. The molecular ions, and fragments, are then separated by the magnetic field according to their m/z ratio and, finally, analyzed.

Other methods of detection are TDC (Thermal Conductivity Detection) and PID (Photo-Ionization Detector).

### 3.3 Other frequently used analysis instruments at a brewery

A brewer does not only measure chemical compounds but also parameters such as the haziness, the color, the bitterness, and foam stability.

- The haziness is measured using a nephelometer. A nephelometer measures the concentration of suspended particles (particulates) in a liquid or a gas colloid by employing a beam of light through a sample and then detecting reflecting light, usually at 90º of the source beam.
- The color intensity is measured using a spectrophotometer (SRM method), at 430 nm.
- The bitterness can be measured using a spectrophotometer or by using a suitable chromatographic method.
- The beer head is important for the mouthfeel, aesthetics, and aroma of the beer. Therefore, foam stability (durability) is assessed observing the time it takes for the foam to drain, i.e. the time required for 50% of the liquid to drain from foam (a 50% reduction in foam volume).

### 3.4 Metabolomics, a future opportunity

A future analysis approach benefiting brewers could be ***metabolomics***, where many metabolic reactions are studied simultaneously. In metabolomics, the focus can be on a detailed study of metabolism (specific metabolic events) during e.g. fermentation, or on a broad unbiased approach (nontargeted) where many simultaneous metabolic events are “recorded”. This is done by determining the amounts and types of produced metabolites and thereby elucidating the chemistry in metabolic processes. A metabolome is, consequently, a complete set (can be from several hundred up to several thousand) of small molecule chemicals present even in an entire organism, a tissue, an organ, a cell, or cellular organelle. We are, thus, able to produce accurate and unique metabolic “fingerprints” from which one can see if e.g. unwanted reactions, such as bacterial contaminations, take place or to monitor yeast cultures for their potential. These investigations demand highly sophisticated analytical instruments, like GC-MS-MS (two mass spectrometers in an array to get a precise structure of the analytes) and NMR (Nuclear Magnetic Resonance Spectroscopy), and additionally, well-educated personnel to master both the analyses and the interpretations of the results. Much of the focus in today’s system biology is laid on the correlation of genetic expression (mRNA gene expression), proteomics, and metabolomics, so that detailed cellular functions can be elucidated. [25]

# 4 ASSESSMENT OF SUITABLE LABORATORY EQUIPMENT FOR A SMALL BREWERY

### 4.1 Assessment of the necessity of laboratory analyses.

The need for chemical analyses, for a small brewery, might not be obvious especially since the only chemical analysis required by authorities is the ethanol content of the finished beer. However, if the brewery wants to compete for customer using e.g. quality and products having balanced flavors, the need for analysis soon becomes apparent. Large breweries producing beer in millions of liters use predominantly semi-manufactured raw materials that are delivered with analysis results. Small breweries should be able to follow the quality of their raw-materials and the contents of $\alpha$- and $\beta$-acids, carbonyl compounds, proteins, etc. in their final products to be able to compete and expand. At some stage quality control issues, e.g. identification of the reasons for off-flavors, will be important. The ability to maintain a steady production, demands from authorities, and the ability to resolve product development- and quality issues, are key questions in the expansion of the activities.

The acquisition of laboratory equipment should be planned carefully because especially the more accurate analysis instruments are expensive, especially when purchased as new. The decision to implement a new analysis method does not only mean a monetary investment but it also leads to investments in the form of e.g. training and possibly new recruitments.

The EBC has listed more than 240 analytical laboratory methods (Analytica-EBC, http://www.analytica-ebc.com/), covering raw material analyses, process parameters, and packaged products, to co-ordinate and standardize used analyses in Europe. The analysis method descriptions were written and harmonized with the ISO 78/2-, and ISO 5725 standards.

One option for the brewer is to compose a list of analysis methods that can analyze the most important compounds in beer and raw materials. This data can then be used to organize and prioritize the most useful compounds to be analyzed and analysis methods to be implemented. Below is a list focusing on analysis methods that require (for a small brewery) a relatively large investment, omitting the most simple and inexpensive methods (**Table 2**).

**Table 2. Suitable analysis methods for significant beer flavor compounds.**

| Analysis method and detection | Analytes | Type of compound |
|---|---|---|
| **Spectrophotometric methods** | Bitterness, color, FAN, total polyphenols, diacetyl | Compounds from hops and malt |
| **HPLC** | | |
| UV-Vis (Ultra Violet and Visible light) | alpha acids | Compounds from hops |
| Ion chromatography | ions | Water analyses |
| RID (Refractive Index) | Carbohydrates | Compounds from malt |
| RF | amino acids | Compounds from malt |
| Electroconductive detection | Organic acids | Compounds from hops |
| MS-MS (Mass spectrometry) | Amino acids | Compounds from malt |
| **GC** | | |
| Sampling using headspace | Diacetyl and VDK | Volatiles |
| FID (Flame Ionization Detection) | Alcohols, Esters, Aldehydes | Volatiles and semi-volatiles |
| MS | Amino acid derivatives | Compounds from malt |
| Sulphur detectors (FPD, PFPD, SCD) | $H_2S$, Mercaptans, $SO_2$, $(CH_3)_2S$ (DMS) | Off-flavors |

When the analyses are arranged in order of priority, the assessment of the required investment is relatively simple.

**Table 3. Analysis methods arranged according to priority.**

| Compound type | Analytes | Analysis method, sampling and detection |
|---|---|---|
| **Vital for product development and quality** | Amino- and alpha acids, proteins, VDK, diacetyl and water components | **GC**, and **HPLC systems using headspace- or SPME sampling**, with UV-Vis-, MS-MS-, MS-, RI- electro conductive-, ion-and FID detectors, (and **spectrophotometry)** |
| **Important for flavor components originating from malt or hops** | Carbohydrates, amino acids and their derivatives, bitterness- and color affecting agents, organic acids, amino acids, total polyphenols, sulphur containing compounds | **GC**, and **HPLC systems using headspace- or SPME sampling**, with UV-Vis, RI, MS, MS-MS, electro conductive, and FID detectors, (and **spectrophotometry)** |
| **Harmful** | $H_2S$, Mercaptans, SO2, DMS | GC with sulphur detectors |

According to the brief survey above, the most useful analysis systems for brewers would be based on chromatographic methods and preferentially MS detection. The apparent advantages with these methods are their accuracy, speed, and versatility. However,

most small breweries could find it economically difficult to purchase sophisticated instruments. Therefore, a financial plan should be made where investment costs are subjected to a sensitivity analysis towards e.g. labor costs, flexible- and fixed costs, or utilization rate.

### 4.2 Experimental setup for the investment calculations

In the present experimental setup, a small brewery is planning to start a laboratory for quality control and product development purposes. The business idea includes selling analyses to third parties, to shorten the loan payback time. The needed funds will be borrowed and the estimated utility time is 6 years. The critical issue is the size of the investment with respect to incomes and the time it takes to repay the loan. The payback time was estimated to be 6 years, at a maximum. According to the present survey outlined in chapters 3 and 4, the most useful analysis instruments seem to be a spectrophotometer, a HPLC, and a GC using mass spectrometric detection (or FID). These instruments (HPLC and GC) are, however, very expensive especially if purchased as new. The budgets presented below might therefore be too low, and the instruments may have to be acquired as secondhand.

***Granted funds for investments (€):***

**A. Investments with a small budget.** **Loan sum: 20.000 €**

Includes basic laboratory equipment and a *spectrophotometer*. The incomes and costs are given on a yearly basis and calculated from approximations made for only spectrophotometric analyses.

Additional flexible costs, specific for case A: 500 € / month.

**B. Investments with an average budget.** **Loan sum: 40.000 €**

Includes basic laboratory equipment together with a *spectrophotometer* and an *HPLC*. The incomes and costs are given on a yearly basis and calculated from approximations made for HPLC- and spectrophotometric analyses.

Additional flexible costs, specific for case B: 800 € / month.

**C. Investments with a large budget.** **Loan sum: 70.000 €**

Includes basic laboratory equipment together with a *spectrophotometer* and a *GC-MS*. All incomes and costs are given on a yearly basis and calculated from approximations made for GC-MS- and spectrophotometric analyses.

Additional flexible costs, specific for case C: 1400 € / month.

The funds needed to start a simple laboratory do not have to be large (~20 000 €) but then the amount and quality of acquired information is relatively restricted and shallow. Spectrophotometric analysis can show the color and the compound level (e.g. µg/ml) of compounds but not details e.g. individual- amino acids, carbohydrates or carbonyl compounds. On the other hand, a large investment (~70 000 €) would give the laboratory an instrument that would enable the analysis of most compounds, at a high accuracy. A third option would be a sum in between that would give a reasonably good analysis equipment, but not as accurate and efficient as "option C". To aid in the choice between these options, income- and cost evaluations were made and this data was then used to perform investment calculations. The investment options "B", and "C", included also option "A" (a spectrophotometer is the only instrument by which color can be measured), affecting the usage of the main instruments in resp. scenario. The approximated analysis time for the main instruments in scenarios "B" and "C" (a HPLC or a GC-MS, resp.), was 70% and the remaining 30% analysis time was allocated to the use of the spectrophotometer.

### 4.2.1 Items of expenditures

Certain investments must be made, for all chosen cases (outlined below) on basic laboratory equipment. These investments include consumables (e.g. glassware, pipette tips, parafilm, syringes, protecting gloves, test tubes, etc.), pipettes, magnetic stirrers, fume boards, laboratory centrifuges, basic chemicals, refrigerators, etc. The items of expenditures, and incomes, for cases A, B, and C, are outlined below.

Fixed costs: ~48 500 € / year

Flexible costs: ~8 700 € / year

**Total fixed- and flexible costs: ~57 200 € / year**

The major part (85%) of the costs are fixed costs, such as salaries, book keeping, marketing, rent (+ water and electricity), insurance fees, etc. The flexible costs (e.g. consumables, development, and subcontracting) constitute only ~15% of the expenses and will therefore not have a decisive impact on the profitability of the laboratory.

The calculations of costs and prices were first approximated by attempts to follow the principles of ***ABC*** (Activity Based Costing). At this initial stage the cost structure was, however, simple and the accurate demand of resources (e.g. time for the various analyses) was not correctly known. This approach would therefore have complicated unnecessarily the calculations and therefore ***process costing*** was used instead to price the

analyses. The total yearly costs were, thus, divided with the total hours of laboratory work (sample preparation, analysis time, interpretation of results, and cleaning).

The working hours in one month were calculated to be 160 h (8 h * 5 days * 4 weeks) of which ~35% must be assigned to office work (activity planning + bureaucracy), leaving the rest (~65%) for laboratory work (sample preparation, analysis time, interpretation of results, and cleaning). The cost differences for the different analyses cases (A, B, and C) were adjusted using a specific additional flexible cost (overhead). The analysis costs, per hour, were derived by dividing the total costs (in one year) with the total working hours (in one year) so that case specific costs were considered.

The costs for using and maintaining a laboratory was, according to calculations:

**A**: ~56 -, **B**: ~58 -, and **C**: ~63 € / h

### 4.2.2 Incomes

The average analysis prices obtained from the web pages of five commercial laboratories were, seen from a small brewery point of view, high. The prices were usually given according to used analysis time which included normal preparation of the samples (not more elaborate preparations or sampling). The common trend was, as expected, that the price increased with increasing accuracy of the used analysis method.

***The average analysis prices for competitors, per hour, were:***

- Spectrophotometric analysis: 60 €/h
- HPLC analysis: 200 €/h
- Gas chromatographic analyses: 200 - 250 €/h

***Prices (incl. a 20% profit margin), per hour, provided by the new laboratory, were:***

- Spectrophotometric analysis: 104 €/h
- HPLC analysis: 108 €/h
- GC analysis: 115 €/h

### 4.2.3 Investment calculation variables, parameters of interest

In the present investment calculations three (3) set-ups are considered, according to the amount of funds granted for the laboratory investments. The granted funds were denoted as “small”, “average”, and “large”, based purely on appraised costs for the main analysis

instruments (a spectrophotometer, a HPLC, and a GC-MS, cases A, B, and C, resp. See below for details). Additional flexible costs were added to the costs so that they were presumed to be somewhat higher for the cases having more expensive instruments.

The calculations comprise scenarios where the income varied ±10% so that the "Realist" was 100%, the "Optimist" was 110%, and the "Pessimist" was 90% of the estimated income from analysis fees (the resp. number of performed analyses multiplied with 1,1 and 0,9 for the optimist and the pessimist scenarios, respectively). The input data on incomes did not differentiate between the analyses made on own samples and those made for customers.

Initial tests revealed that the ***labor costs*** were, by far, the highest expenditure (64% when the salary was 3000 € / month) for the laboratory. This parameter was therefore studied. Another parameter was the ***rate of utilization***. A substantial part of the laboratory work must be assigned to maintenance, planning (with administration), and development, including quality control and research. In these scenarios the rate of laboratory utilization is especially important since there will be only one person working in the laboratory and the above mentioned issues are vital for the survival of the laboratory. The **optimum share** of time spent at laboratory work was estimated to be **65%** of the total working time. The estimated amount of analyses was approximated to be 65% of the maximum (because the number of customers will, in the beginning, be low).

The calculated interest is used to discount incomes and expenses to their present value, i.e. an internal rate of return (%) is assigned that gives a (net-) present value of zero for the investment. The **internal rate of return** (internal rate of investment) thus shows the profitability of the investment and is thereby an important parameter to follow.

The analysis of the different scenarios (Cases A-C) were made using a MS Excel workbook (Perusinvestointilaskentapohja.xls, version 2.02*) which discounts the invested funds and calculates the repayment time according to current incomes and costs.

* Varis, K., Lecture material from the course: "Financial administration in management", Spring 2017.

# 5 RESULTS AND DISCUSSION

The calculated interest was approximated to be 20% which was also set to be the aimed yield of the investments. The parameters were adjusted to give a realistic representation of the income- and cost structures. In preliminary calculations (results not shown), it was found that an internal rate of return was good when this value was ≥20%.

The calculated price for the most simple scenario (Case A), where a spectrophotometer was purchased, the analyses prices were markedly higher for the planned laboratory than for existing laboratories (104:- and ~60 €/h, resp.). The reason for the expensive analyses was that the prices were calculated according to costs per unit time. This will be a problem because the price difference was so large. However, the calculated prices for the more advanced analyses (Cases B and C) would be ~50% cheaper, when made by the planned laboratory, making the prices very competitive.

## 5.1 Initial calculations

The opening calculations, for the simplest laboratory set-up (Case A), showed that the profit would be small and that the number of sold analyses must be large if the business was to be profitable.

Key variable figures, in the present survey, were:

- Salaries
- Sold analysis hours (capacity)
- Internal rate of interest
- Incomes and expenses

### 5.2 Case A: Investment budget of 20 000 €

An investment of ~20 000 € will give the brewery the ability to perform simple analyses that, although comprising several different types of analyses, are time consuming and give a relatively small profit. Another severe problem, in this case, is that the price of the analyses is much higher compared to those of the competitors. The amount of sold analyses will, therefore, be low (exemplified by the low level of used capacity).

**Annual economic figures:**

| | |
|---|---|
| **Incomes** | **65,47 k€*** |
| Optimist | 72,02 k€* |
| Pessimist | 58,92 k€* |
| Costs | 63,20 k€* |
| Internal rate of return | -7,0% |
| **Repayment period:** | **Not feasible** |

**Used laboratory capacity: 55%**

(*1k€ = 1000€)

Calculations showing the net cash flow (calculated using the "realist" values) and the tri-valued re-payment rate showed that the investment will not pay for itself unless the optimist conditions lasts for at least 2 - 3 years (**Figures 9 a and b**). This scenario of a laboratory setup was also unusable because only 55% of the laboratory time capacity was utilized and the internal rate of return was negative. Thus, these results showed, that spectrophotometric analyses alone are too laborious and expensive bringing in too little incomes to constitute the sole selection of analyses. The salaries constituted ~63% of all costs (data not shown) so a test was made where the salaries were lowered by 20%.

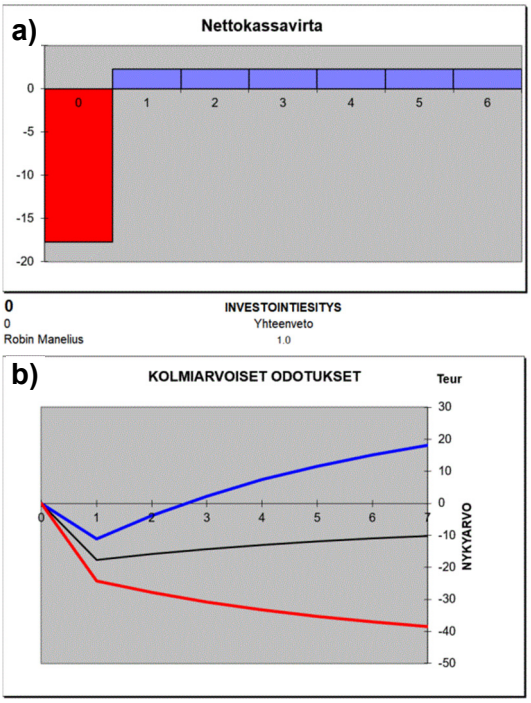



**Figure 9. Case A. Initial calculations on a 20 k€ investment.**

The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

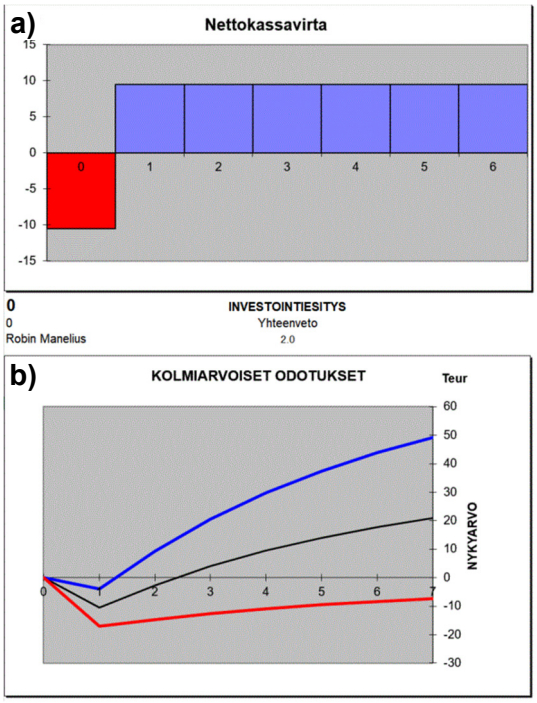



**Figure 10. Case A, scenario with lower salary.**

The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

**Figures 10 a and b** show the drastic effect of a 20% monthly reduction (from 3000 € to 2400 €) of the salary.

**Annual economic figures:**

| | |
|---|---|
| **Incomes** | **65,47 k€*** |
| Optimist | 84,48 k€* |
| Pessimist | 69,12 k€* |
| Costs | 56,00 k€* |
| Internal rate of return | 88% |
| **Repayment period:** | **~2,5 years** |
| **Used laboratory capacity:** | **55%** |

(*1k€ = 1000€)

The realism of the calculations improved significantly for all figures, although the aimed utilization rate of 65% laboratory work (enough customers) time was not met. Furthermore, the salary was far too low after the reduction, making it very difficult to find anyone who would do this demanding work for that salary.

An investment of 20 000 € was, thus, clearly too small to be sustainable.

### 5.3 Case B: Investment budget of 40 000 €

A doubling of the investment budget would allow the acquisition of a HPLC analysis system. The opportunities for flavor component identification and quantification would thereby be significantly improved. The increase in the amount of more detailed analyses thus justifies the increase, by 10%, in used capacity.

**Annual economic figures:**

| | |
|---|---|
| **Incomes** | **80,18 k€*** |
| Optimist | 88,20 k€* |
| Pessimist | 72,17 k€* |
| Costs | 66,80 k€* |
| Internal rate of return | 45% |
| **Repayment period:** | **~3,5 years** |
| **Used laboratory capacity:** | **65%** |

(*1k€ = 1000€)

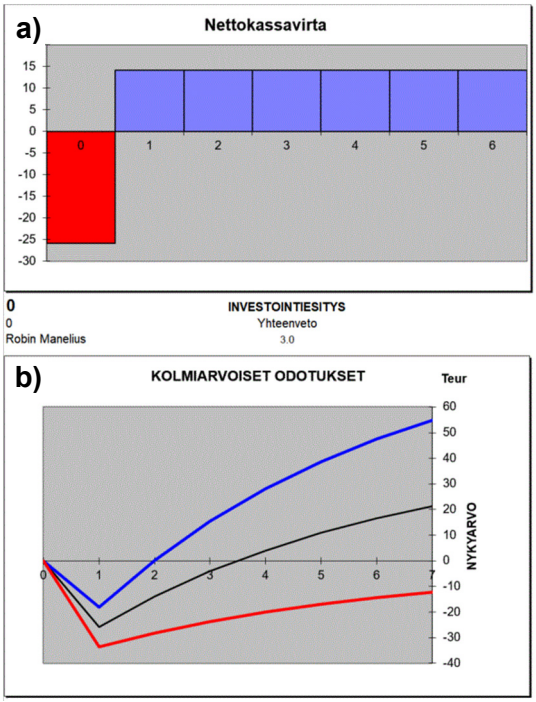



**Figure 11. Case B, a 40 k€ investment.**
The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

The pronounced effect of sold analyses was seen also in this scenario (**Figures 11 a and b**). The realistic investment pay-back time is ~3,5 years and, under optimistic conditions (increase in laboratory analyses by 10%), only ~2 years. The pessimistic conditions (decrease in laboratory analyses by 10%) will not allow payback of the investment, making this scenario somewhat risky. The effect of a salary reduction (-10%), was tested and

this scenario resulted in a reduction of the pay-back time with about one year and an increase in the internal rate of return by almost 30% (results shown in the appendix). These results indicated that although a clear improvement in the profitability (compared to the case in 5.2) was seen, the opportunities to develop the laboratory further for example by employing a laboratory assistant, would be difficult. An investment of this size would perhaps be suitable for a brewery that does not have ambitions to sell analyses to third parties.

### 5.4 Case C: Investment budget of 70 000 €

An investment of 70 000 € (70 k€), on a laboratory is a considerable economical risk for a small brewery. In this scenario, all the hours allocated for flavor component analysis, must be used to give realism to the calculations (results not shown). The profitability was significantly improved when full laboratory capacity was used (**Figures 12 a and b**), showing that there is much potential in this scenario provided that the laboratory has enough customers.

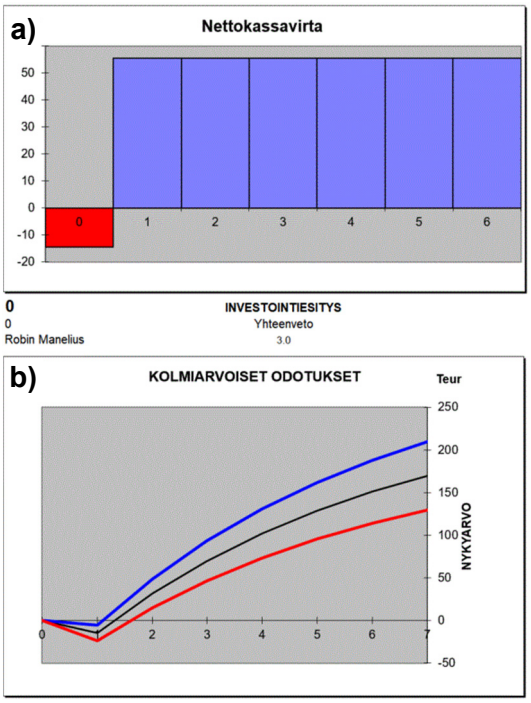



**Figure 12. Case C, a 70 k€ investment.**
The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **123,16** | **k€** |
| Optimist | 135,70 | k€ |
| Pessimist | 111,02 | k€ |
| Costs | 66,80 | k€ |
| Internal rate of return | 380% | |
| **Repayment period:** | **~1,2 years** | |
| **Used laboratory capacity: 100 %** | | |

The loan repayment time was relatively short, only ~1,2 years, and even in the pessimist scenario the repayment time was about 1,5 years. However, the internal rate of return is extremely high (~380%) which is misleading. Nevertheless, the combination of an increase in incomes from analysis time and a more efficient usage of laboratory time has a substantial impact on the profitability. Because of the importance of steady and relatively high incomes, a test was made where the sold laboratory hours were only 80% of the maximum capacity. This test should also clarify the level of vulnerability on the activities, if incomes are low. The laboratory activities must have a chance to expand and

develop and thereby to remain competitive. **Figures 13 a and b** show the influence of a substantial (20%) reduction in the incomes. The repayment period (~4,5 years) was now much longer, but still within acceptable limits.

The internal rate of return was now still high but at the same time realistic. Re-payment calculations showed that a 10% increase in the incomes enhance clearly profitability, and a further lowering of incomes would make the activities unprofitable (the optimist- and pessimist scenarios, resp., **Fig. 13 b**).

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **98,69** | **k€** |
| Optimist | 108,56 | k€ |
| Pessimist | 88,82 | k€ |
| Costs | 66,80 | k€ |
| Internal rate of return | 35% | |
| **Repayment period:** | **~4,5 years** | |
| **Used laboratory capacity: 80%** | | |

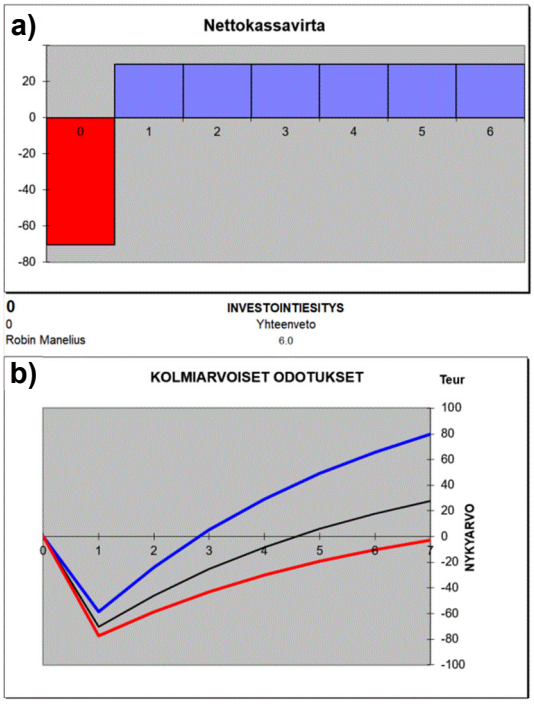



**Figure 13. A 70 k€ investment and 80% laboratory usage efficiency.**
The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

The net-cash flow calculations (**Figs. 12 a and 13 a**) showed, thus, that the laboratory would have a much more solid economy with this higher investment than in the other scenarios (**Figs. 9 a, 10 a, and 11a**). One drawback in the scenarios, so far, has been that the calculations have not shown any possibilities for expanding the activities (e.g. by employing laboratory personnel). The net -cash flow prediction was, after the first year, ~8 - 13 k€ in the scenarios where the investment was 20 – 40 k€ and > 50 k€, when the investment was 70 k€ (**Fig. 12 a**). The calculations showed a net -cash flow ~25 k€ (after the first year) even after the laboratory capacity usage was lowered to 80% of the optimal (**Fig. 13 a**). These figures gave a reason to test possibilities for the employment of a laboratory assistant. The incomes, however, were not high enough to enable further hiring, if the employment was done already at the first year of operation (results not shown). **Figures 14 a, and b**, show the economical prediction in the case that an employment was made after the two first years of operation and the laboratory usage is at its full potential (100%). The prediction was optimistic in

that, after the greater part of the investment was re-payed, the present value of the investment increased at a steady rate of ~8 k€ / year.

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **123,4** | **k€** |
| Optimist | 135,7 | k€ |
| Pessimist | 111,0 | k€ |
| Costs | 106,8 | k€ |
| Internal rate of return | 70% | |
| **Repayment period:** | **~2,0 years** | |
| **Used laboratory capacity: 100%** | | |

The calculation, thus showed that the present scenario allowed more options for development and expansion. It seemed obvious that a laboratory needs, for expansion, more performed analyses and a broader range of analyses, as seen from previous calculations (Cases A and B).

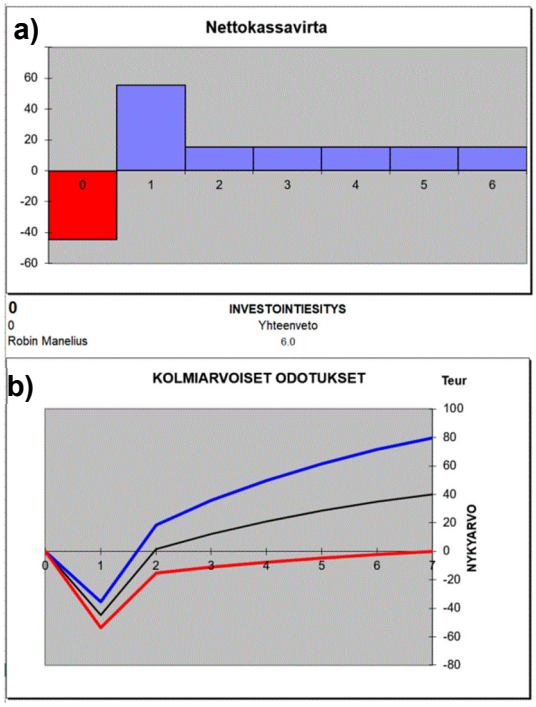



**Figure 14. A 70 k€ investment and employment after the second year.**
The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

In the last scenario (holding time: 10 years), the initial investment was a GC equipped with a mass spectrophotometric detector (70 k€).

- After 2 years from start, the laboratory will employ a laboratory assistant (salary: 2200 €/month + 880 €/month social security costs) increasing the overall share (potential) of laboratory work with ~30 hours per week (from 26 to 35 h / week, an increase by >300%) of which ~80% is expected to be used. The effect of this measure would be that the incomes are expected to increase by ~53% and the price per analysis hour will increase (by ~46%).
- A HPLC will be acquired after 5 years (an investment of 30 k€ and will be utilized 10% of the used laboratory time) increasing the amount and type of analyses (the utilized analysis time) to ~90% of the used laboratory capacity. The incomes were estimated to increase further by ~14%.

The results were encouraging in that the repayment time was short for all situations (the realist, optimist, and pessimist), allowing a repayment and recovery from increased expenses, within a period of ~2 – 3,5 years (**Figs. 15 a and b**).

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **202,3** | **k€** |
| Optimist | 222,5 | k€ |
| Pessimist | 182,1 | k€ |
| Costs | 114,0 | k€ |
| Internal rate of return | 99,6% | |
| **Repayment period:** | **~2 – 3,5 years** | |
| **Used laboratory capacity: 80%** | | |

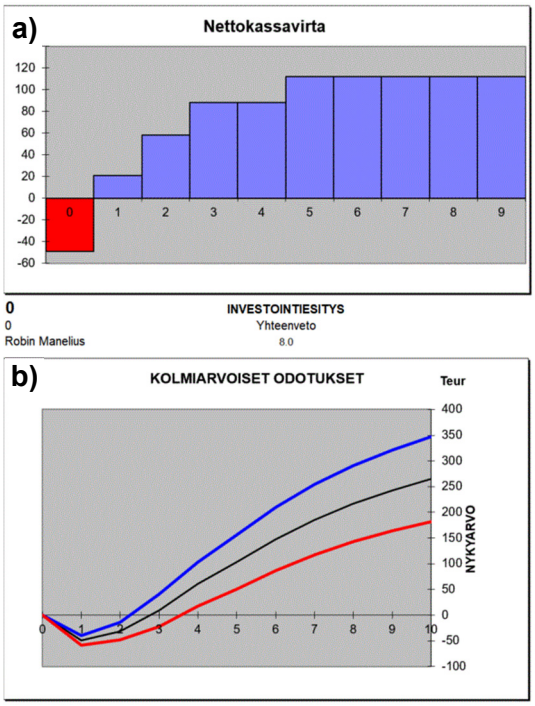



**Figure 15. A combination of investments.**

The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

The price of the analysis time was not raised at any time, during the present experimental setup, giving the impression of a robust and profitable scenario. The internal rate of return was, however, very high (~100%) which gave a reason to examine the results critically. There could, nonetheless, be a risk that the incomes were calculated too optimistically and that the real quantity of incomes could be much less. This was tested by lowering the analysis prices with 10%, and 20%. These test conditions showed that the internal rate of return was lowered to 68%, and 43% (income decrease of 10% and 20%, resp.) and the repayment period was prolonged to ~3,5 - 5 years (income decrease of 10% and 20%, resp.). The net cash flow was lowered with ~10 k€ annually for every 10% decrease and the activities remained profitable until the income decrease was 30% (results shown in the appendix).

To test the reliability of the present experimental setup, a test was made where a pension insurance premium (20 % of the salary, i.e. 7,2 k€ / year) was added to the costs, reducing the demand for a high internal rate of return. The rates of interest were, at the time of writing, low. The calculated interest was thus estimated according to WACC (weighted average cost of capital), which at the moment, was estimated to be 1,8% (interest of liabilities ~0,8% + 1,0% marginal interest rate of the bank). However, the interest rates might increase during the loan period (6 years), which should be considered, and therefore an interest rate of 3,5% was chosen (results shown in the appendix). This test did not show any marked worsening of the economy compared to those seen in **Figures 15 a and b**.

In the final test, the laboratory efficiency rate was lowered until the activities became unprofitable (at ~62% of used laboratory capacity). The conditions were as above including a pension insurance premium and a calculated interest according to WACC. The results can be seen from **Figures 16 a and b**.

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **156,8** | **k€** |
| Optimist | 172,5 | k€ |
| Pessimist | 141,1 | k€ |
| Costs | 121,2 | k€ |
| Internal rate of return | 29,7% | |
| **Repayment period:** | **~5,5 years** | |
| **Used laboratory capacity: 62%** | | |

In this test the internal rate of return was still ~30% even when the used laboratory capacity was only 62%, confirming the earlier tests (Figs 13 and “Case C: 20% decrease in incomes from sold analyses” in the appendix) where incomes were lowered. The repayment time and in this test, was ~5,5 years which was still within the acceptable time frame, showing the potential of this laboratory set-up (case C).

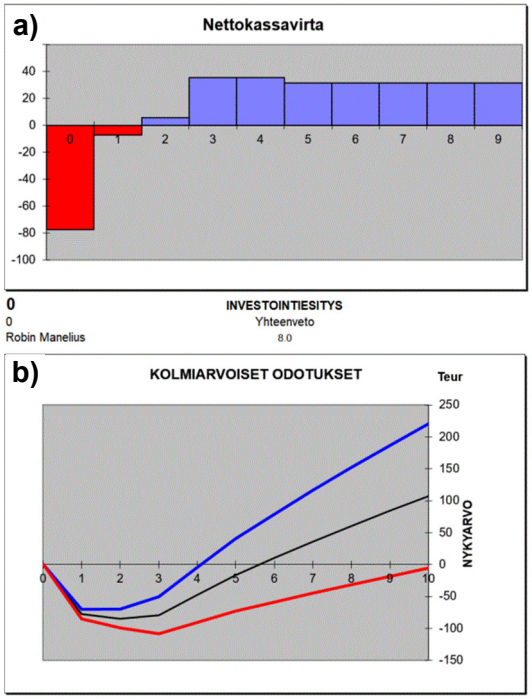



**Figure 16. A test of tolerance towards a low market demand for laboratory analyses.**

The figure shows the net cash flow, k€/year (**a**) and tri-valued re-payment time calculations (**b**).

# 6 CONCLUSIONS

Beer and the raw materials for brewing contain a vast number of components that affect the flavor of a brew. There are also many flavor components that should not be present in beer and which must be identified and traced so that corrective measures can be taken. The origin of off-flavors can be clarified provided that suitable analysis equipment is at hand. There are multiple motives for chemical analysis of beer. The motives can be based on quality control (on raw materials as well as on the intermediate or final brew), product development, or purely on business. Quality control analyses can be used to track faulty raw materials as well as the origin of problems in the brewing process. Modern analysis instruments provide many opportunities but they also demand a lot of education and practice. Planning of analyses should therefore be thorough and further education should be included in the laboratory development plan. Most flavor components in beer are different carbonyl species and amino acid derived compounds. Their identification and quantification should be the goal of any laboratory that has invested in analysis equipment such as HPLC or gas chromatography. Other interesting molecules are sulphur containing compounds and polyphenols.

Analysis instruments that is usually used to accomplish the analyses are HPLC or GC (with MS- or FID). These instruments are selective enough to separate and quantify the molecular species provided that suitable standards can be found. New flavor species can be identified if mass spectrometry is used as detection method. Beer can also be analyzed using simpler analysis methods, such as spectrophotometry. These methods show, however, only some components (often after laborious preparations) and are therefore more suitable for routine quality control analyses, e.g. for quantifying beer color and bitterness.

Founding a laboratory is expensive and the planning process should therefore be thorough and cover all areas from analyte identification to economic planning. Economic planning is essential when the investments are large and no previous experience has been gathered about incomes, demand for analyses, or quality requirements. Some of the analysis instruments are so expensive that second hand equipment should be considered although there are risks (e.g. guarantee issues) with used instruments. Tests were therefore made, using economic models where the investment (costs and in-

comes), covering a period of six years, was discounted to present value and the repayment time of the loan(s) was calculated. The tests indicated strongly that the invested funds can well be rather large provided that the analysis results are informative, and that the largest expenses (salaries) are under control. A smaller investment (20 000 €) was enough to acquire simpler analysis equipment but it is unlikely that customers would pay enough for the analyses, so that the laboratory could develop within a reasonable period of time. The analysis prices (price of analysis time) and analysis time utilization rate were thus the most important parameters in these calculations. The present survey showed that analysis quality is vital for the feasibility of a laboratory, especially if the analysis time will be sold to third parties. A substantial increase in analysis capacity can be achieved by employing personnel to the laboratory. Vital issues, such as analysis- and economic planning can now be made more thoroughly while the analysis equipment is in productive use. The survey showed also that the largest economical risk is a situation where the laboratory does not get enough analysis orders (below ~60% of maximum laboratory analysis capacity) to cover the costs of the laboratory. This limit (~60% of the laboratory capacity), as the minimum usage of laboratory, should therefore be considered a guideline for market- and feasibility analysis.

# 7 APPENDIX

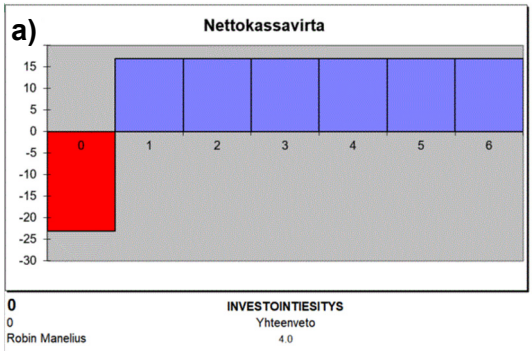
## Investment calculations:

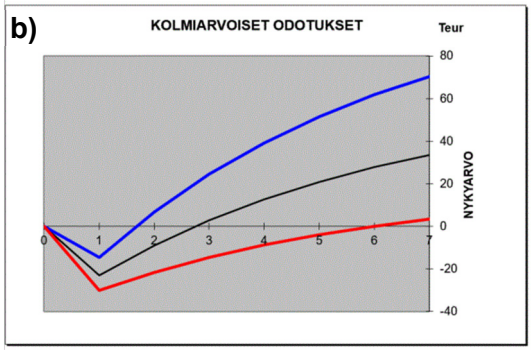
**Case B: Effect of a 10% salary reduction.**

**Annual economic figures:**

| | |
|---|---|
| **Incomes** | **80,18 k€** |
| Optimist | 88,20 k€ |
| Pessimist | 72,17 k€ |
| Costs | 63,20 k€ |
| Internal rate of return | 70,8 % |
| **Repayment period:** | **~2,7 years** |
| **% used laboratory capacity:** | **65%** |

Case B, net cash flow (a) and tri-valued re-payment time calculations (b). The effect of a 10% salary reduction.



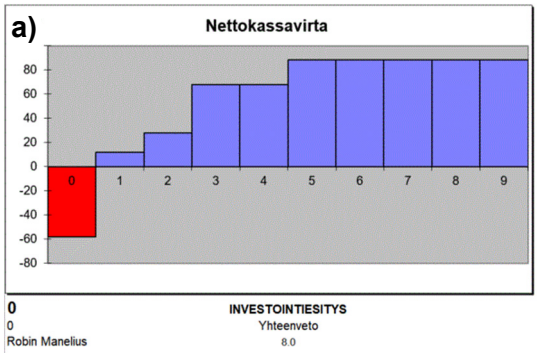



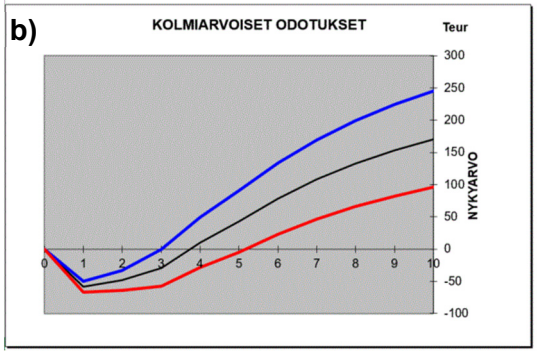

**Case C: 10% decrease in incomes from sold analyses.**

**Annual economic figures:**

| | |
|---|---|
| **Incomes** | **182,0 k€** |
| Optimist | 200,3 k€ |
| Pessimist | 163,9 k€ |
| Costs | 114,0 k€ |
| Internal rate of return | 65,2% |
| **Repayment period:** | **~3 - 5 years** |
| **% used laboratory capacity:** | **80%** |

Case C, net cash flow (a) and tri-valued re-payment time calculations (b). The effect of a 10% analysis price reduction.



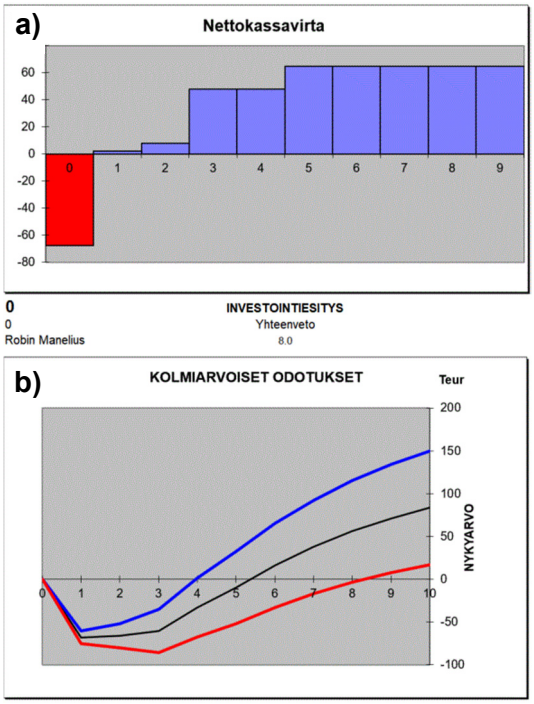



**Case C: 20% decrease in incomes from sold analyses.**

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **161,9** | **k€** |
| Optimist | 178,0 | k€ |
| Pessimist | 145,7 | k€ |
| Costs | 114,0 | k€ |
| Internal rate of return | 40,9% | |
| **Repayment period:** | **~4 - 8 years** | |
| **% used laboratory capacity:** | **80%** | |

Case C, net cash flow (a) and tri-valued re-payment time calculations (b). The effect of a 20% analysis price reduction.

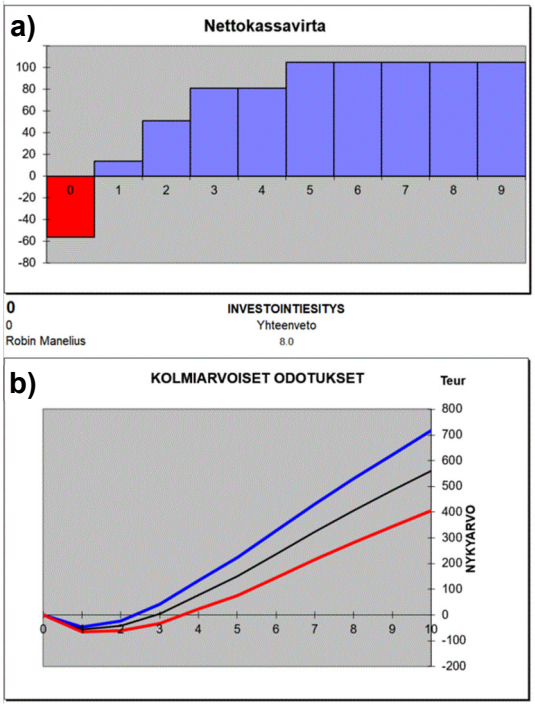



**Case C: An addition of a pension insurance premium (20% of the salary) to the costs and a concurrent reduction (to 3,5%) of the calculated interest.**

**Annual economic figures:**

| | | |
|---|---|---|
| **Incomes** | **202,3** | **k€** |
| Optimist | 222,5 | k€ |
| Pessimist | 182,1 | k€ |
| Costs | 121,2 | k€ |
| Internal rate of return | 81% | |
| **Repayment period:** | **~3 years** | |
| **% used laboratory capacity:** | **80%** | |

Case C, net cash flow (a) and tri-valued re-payment time calculations (b). The effect of a pension insurance premium, of 20% of the salary.



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