Universidade do Minho
Escola de Engenharia

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Application of hydrodynamic cavitation in brewing

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In honour of my dear father. Always has been an example and a source of inspiration to me, and forever will be.

STATEMENT OF INTEGRITY

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

RESUMO

A cerveja é uma bebida muito popular em todo o mundo e que tem uma grande história até aos dias de hoje, sendo a terceira bebida mais consumida em todo o mundo. É obtida por fermentação alcoólica, utilizando leveduras geralmente do género *Saccharomyces*, num mosto preparado a partir de malte de cereais, no qual foram adicionadas flores de lúpulo ou produtos de lúpulo e água potável.

A indústria da cerveja, como a conhecemos, é o resultado de um longo desenvolvimento. A forma de produzir cerveja não é muito variável, pelo que esta indústria se baseia na criatividade para inovar nesta área. Além disso, atualmente várias investigações têm sido realizadas com vista a inovar tecnologicamente o processo. Uma das tecnologias mais investigadas neste campo, e o foco central deste estudo é a aplicação da cavitação hidrodinâmica no fabrico de cerveja, mais precisamente durante a fase de ebulição do mosto, a fim de poupar energia no processo. O fenómeno de cavitação consiste (com auxílio de maquinaria) na formação e colapso de pequenas bolhas de vapor que geram grandes quantidades de energia no meio.

O principal objetivo deste estudo é fazer uso da tecnologia na ebulição do mosto, e compreender as condições que otimizam a isomerização dos ácidos alfa, bem como compreender se é possível reduzir os níveis de glúten na cerveja.

As experiências foram realizadas num wort kettle com um volume de 50 L. Foram testadas diferentes temperaturas num intervalo de 70 a 90 °C, bem como diferentes números de cavitação sendo aplicados num intervalo de 0.062 a 0.15. Além disso, foi realizada uma experiência com pellets de lúpulo ao invés de extrato de lúpulo. Foram também realizadas experiências a 70 e 100 °C (temperatura de ebulição) sem cavitação, a fim de comparar os resultados com a utilização da tecnologia.

A isomerização mais elevada (63 %) foi obtida a 90 °C aplicando um número de cavitação de 0.062. Esta foi superior à isomerização obtida para a experiência de ebulição tradicional (53.7 %). Os resultados mostraram que a temperatura de 90 °C, independentemente do número de cavitação, parece muito promissora e resulta numa melhor isomerização, ou pelo menos comparável à ebulição tradicional. Relativamente aos resultados de glúten, a cavitação hidrodinâmica mostrou diminuir a concentração de gliadina (proteína de glúten) no meio. No início da experiência foi detetada uma concentração de 65.3 ± 9.5 mg/L de gliadina enquanto no final 49.7 ± 7.2 mg/L.

Palavras-chave: Cavitação hidrodinâmica, Ebulição do mosto, Isomerização, Processo cervejeiro

ABSTRACT

Beer is a very popular drink around the world, a beverage that has a great history until today, being the third most consumed drink in the entire world. It is obtained by alcoholic fermentation, using usually selected yeasts of the *Saccharomyces* genus, into a wort prepared from cereal malt, in which flowers of hops or hop products and potable water have been added.

The beer industry as we know is the result of long development. The way of producing beer is not very variable, so this industry is based on creativity to innovate in this area. Furthermore, research is currently being carried out in order to technologically innovate the process. One of the most in-depth technologies in this field and the central focus of this study is the application of hydrodynamic cavitation in brewing, more precisely during the wort boiling stage, in order to save energy in the process. The cavitation phenomenon consists of (with support of machinery) the formation and collapse of small steam bubbles generating large amounts of energy in the medium.

The main goal of this study is to make use of the application of the technology in wort boiling and understand the conditions that optimise the isomerisation of bitter alpha acids, as well as to understand if it is possible to reduce the levels of gluten in beer.

The experiments were performed in a wort kettle with a volume of 50l. Different temperatures were tested in a range from 70 to 90 °C, as well as different cavitation numbers being applied in a range from 0.062 to 0.15. Furthermore, an experiment with hop pellets instead of hop extract was performed. Besides that, experiments at 70 and 100 °C (boiling temperature) without cavitation were also performed in order to compare the results with the use of the technology.

The highest isomerization (63 %) was obtained at 90 °C applying 0.062 cavitation number. This was higher than the isomerization obtained for the experiment of traditional boiling (53.7 %). The results have shown that the use of 90 °C, regardless the cavitation number, seem very promising and results in isomerization better or comparable to traditional boiling. Regarding the gluten results, hydrodynamic cavitation has shown to decrease the concentration of gliadin (protein of gluten) in the medium. At the beginning of the experiment it was detected a concentration of 65.3 ± 9.5 mg/L gliadin while at the end 49.7 ± 7.2 mg/L.

Keywords: Brewing, Isomerization, Hydrodynamic cavitation, Wort boiling

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List of Abbreviations and Acronyms

% - percentage

˚C – Celsius

atm – atmosphere (pressure)

CN - Cavitation number

HC - Hydrodynamic cavitation

hl – hectoliter

HPLC - High-performance liquid chromatography

IBU – International Bitterness Units

K – Kelvin

kg – kilogram

kHz – kilohertz

KW – kilowatt

L – liter

mg - miligram

MhL – million hectloliter

MHz –megahertz

min – minute

MJ - megajoule

mm – milimeter

RIBM - Research Institute of Brewing and Malting

UV - Ultraviolet

1. FRAMEWORK

1.1. Research Institute of Brewing and Malting (Prague)

The current study was carried out in the Research Institute of Brewing and Malting (RIBM), located in Prague and Brno. This Institute was founded in 1887 and since 1994 it has been a joint-stock company. It is one of the oldest scientific research institutes in the Central European region.

Figure 1 - Research Institute of brewing and Malting

Its main objective is to gather new knowledge in this area and promote its practical applications. It plays an important part in the development of new technologies aimed at increasing production efficiency and quality while preserving all attributes of the Czech way of beer production and its specific properties (Beer Research, 2018).

1.1. Contextualisation and objectives

Beer is a very popular drink around the world, a beverage that has a great history, with the first mentions of its consumption dating back to 2800 BC in the Mesopotamian area, from where it later expanded into Egypt (Kunze, 2004a). However, it is believed that its consumption occurred as early as the middle of 9000 BC (Brányik & Pires, 2015a).

In the today basis, beer is the third most consumed drink in the entire world after water and tea, being the most consumed alcoholic drink (Kunze, 2004a).

According to the Portuguese legislation, Portaria nº 91/2022, of February 9th, the definition of beer

stands for a beverage obtained by alcoholic fermentation, using selected yeasts of the *Saccharomyces* genus, from a wort prepared from cereal malt, mainly barley, and other starchy or sugary raw materials, to which flowers of hops or hop products and potable water have been added.

According to *The brewers of Europe* (2021), beer production and consumption in the European Union decreased slightly from 2019 (402 MhL) to 2020 (341 MhL), which is justified due to the pandemic situation of covid-19. According to the same source, Germany is the most important country in beer production, standing out from other producers with values of 87 MhL, and it is also the country with the highest total beer consumption in the European Union.

Portugal and the Czech Republic, although demographically very similar, are considerably different in this sector. Portugal is a more recent country in the beer industry, with less tradition in this sector when compared to the Czechs, even because Portugal is situated ıthe south of Europe, characterized for wine production which is also more traditional among the Portuguese. In the other hand, the Czech Republic is a big country in the beer industry, being one of the most iconic nations in this sector with a big history.

Portugal has been slowly increasing both in the production and consumption of beer, however, being the populations of the two countries very alike, it is possible to verify that the industry is more explored in the Czech Republic. They have currently 599 breweries in the asset, contrasting with the 120 from Portugal (data from 2020). Production and consumption levels are also very diverse, with the Czechs producing around 20 MhL of beer and Portugal goes no further than 7 MhL, being a huge difference between the two. Also, the consumption per capita of beer is 46 L/year in Portugal and 135 L/year in the Czech Republic, which leads this ranking among the countries in the European Union. Portugal also stands out because 60 % of what it produces is for on-trade (bars, restaurants, hotels instead of supermarkets), the highest percentage among European Union producing countries. (The brewers of Europe, 2021)

With this considerable difference, it is assumable that in the Czech Republic and at the center of Europe in general, the progresses in this industry are bigger, and the investments in new technologies and new research in the sector are also more common in this region of Europe.

The beer industry as we know is the result of many years of development. The way of producing beer is very traditional, so this industry is based on creativity to innovate in this area which may bring different offers to the consumer, helping the growth of different beer styles. Furthermore, research is currently being carried out in order to technologically innovate the process. One of the most in-depth technologies

in this field with plenty of research, by Albanese & Meneguzzo (2019) and many others, and the central focus of this study is the application of hydrodynamic cavitation in the process.

This investigation has the main goal to study and optimize the effect of hydrodynamic cavitation in wort boiling and see the effects on the isomerization of alpha-acids, reduction of beer gluten content, also with the possibility of saving energy in the process.

2. INTRODUCTION

2.1. Beer raw materials

For beer production it is necessary to assemble the raw materials and all the processes to obtain the final product. Those topics will be discussed in the following points.

Beer production requires essentially four raw materials: malt, yeast, water and hops. The quality of these ingredients is decisive for the quality of the final beer (Kunze, 2004a).

2.1.1. Malt

Malt is one of the most important ingredients when it comes to the production of beer. Usually, barley is the cereal grain that is used the most, however, wheat and sorghum are also malted. Besides that, small amounts of rye, oats and millets can also be used (Briggs et al., 2004a).

Barley has in its composition a significant amount of carbohydrates such as cellulose, hemicellulose, gums, sugars and mainly starch. This raw material has a high starch content, which is important as it will serve as a substrate for the yeast in the medium. Before its use in the brewery, the barley must first be converted into malt (Kunze, 2004a). Malt basically contributes with proteins (including gluten proteins) responsible for beer foam and sugars essential for fermentation (Cela et al., 2020).

There are many types of barley, and its use depends on which beer is being produced. The process may use, barleys which are more roasted, or even with a higher sugar content, which will logically have different effects on the final beer. Figure 2 shows a representation of how the type of malt used in the process can vary (Kunze, 2004a).

Figure 2 - Different types of barley (Craft Beer & Brewing (2020).

The product of interest in barley is the reproductive parts (seeds) of the plant, known as grains or kernels, and are displayed on the ears of the plants (Figure 2). Depending on the species of the barley, the plant will expose one or more kernel per node of the ear. The fewer the kernels per node, the stronger in starch they will be (Brányik & Pires, 2015a).

Barley can be split in two types: winter barleys and spring barleys. Spring barleys have higher amounts of starch, which makes them more suitable for brewing purposes. Also, this type of barleys has been the focus of regular attempts to improve their brewing quality over the years (Kunze, 2004a).

2.1.2. Yeast

Yeast is a fundamental ingredient for beer production and the wort represents a rich source of nutrients for it. This unicellular microorganism uses the sugars in the wort, and through fermentation, transform them into alcohol and CO_2, so yeast has a fundamental role in order to obtain the final beer (Russel, 2018).

In brewing, yeasts of *Saccharomyces* are most often used and that is because it allows not only to produce alcohol through its metabolism, but also impacts the final taste and the different characteristics of the beer (Kunze, 2004a).

Contrasting other raw materials, yeast is a living organism, and so it is necessary to have a good knowledge of their structures and composition, their metabolism and their growth, in order to avoid stress conditions. In terms of its composition, yeasts have a large percentage of proteins and carbohydrates, containing also fats and minerals (Russel, 2018).

In the brewing process there are basically two types of yeasts that are used: the top fermenting yeasts (*Saccaromyces cerevisiae*) that produce *Ale beers*, and bottom fermenting yeasts (*S. pastorianus,* previously labelled of *S. carlsbergensis*) that produces *Lager* beers. These two strains are very distinctive in terms of genomic but mainly physiological, and fermentation wise. Depending on the type of yeast used, it will originate different types of beer (Kunze, 2004a; Russel, 2018).

2.1.3. Water

Beer production presupposes the use of large amounts of water, making this raw material, in terms of quantity, the most important one. Water makes more than 90 % of beer, and so, it is important to look at it as a crucial raw material and understand that different beer styles are influenced by the composition of water (Eumann, 2006).

Therefore, the quality of the water for brewing must be high and normally it is determined by legislation. It must fulfil with the drinking water regulations, concerning the quality of drinking water whether in sensory, physiochemical, microbiological and chemical terms (Kunze, 2004a).

Water has in its constitution dissociated ions. Water has a neutral pH because the number of hydrogen ions is the same as the number of hydroxyl ions. Some of these ions react with compounds of malt during beer processing, and this results in acidity changes during beer production, thus affecting enzymatic and non-enzymatic reactions. Having that in account, it is important to control this parameter (Kunze, 2004a).

To guarantee water of high quality to the beer process, apart from the legal requirements, its treatment is in many cases necessary. The use of treatments such as sterilisation by filtration, UV radiation, sterilisation by ozone, by chlorination and by chlorine dioxide helps maintaining water free from microorganisms. Other treatments such as flocculation or reverse osmosis also have the capacity of microbial removal, but these processes are primarily used for other purposes (Kunze, 2004a).

Nowadays, the demineralization of water is a common procedure, and then, the compositions of the process streams tend to be adjusted to meet the different process requirements (Briggs et al., 2004b).

2.1.4. Hops

Hop (*Humulus lupulus L.*), represented in Figure 3, is a perennial diecious climbing plant which belongs to the hemp family. For brewery purposes it is the inflorescences of the female plant that are used in the process. It is basically responsible to supply the bitterness and some aroma components to the beer, because among all the substances, it contains bitter resins and essential oils. Besides that, hops are also important because they contribute to the stability of the foam and inhibit the development of microorganisms in beer. (Kunimune & Shellhammer, 2008).

Figure 3 - Hop plant (Rhoades, 2021).

In brewing there are three ways to apply hops in the process: Through hop pellets, hop extracts or natural hops as shown in Figure 4. Nowadays, breweries tend to use more hop pellets and hop extracts in the process as these products can yield constant levels of bitterness and aroma in beer, their storage can be very long and their use does not require the use of a hop separator (Kunze, 2004a).

Figure 4 - Different type of extract used in brewing (Anonymous & Berry, 2022) adapted.

Czech Republic is one of the most popular countries when it comes to the production of hops in the entire world. Areas such as Sanz region, Auscha and Tirschitz are great hop growing locations. Only aroma varieties are grown. The Saazer hops with an average alfa-acid content of 4.0 % is still viewed as one of the best aroma hops (Kunze, 2004a).

2.1.4.1. Hops Chemistry

According to their physicochemical properties, the secondary metabolites from hops are categorised into three main fractions: the hop resins, the hop oils and hop polyphenols.

HOP RESINS

Hop resins are characterised by their solubility in cold methanol and diethyl ether. They can be classified as hard or soft resins depending on their solubility in hexane. The hard resins are the ones that are insoluble in hexane, with the major constituent of it being xanthohumol. These, have a minor impact on the final beer (Steenackers et al., 2015).

Soft resins are soluble in hexane and mainly contain hop α -acids and hop β- acids, represented in Figure 5 (Steenackers et al., 2015).

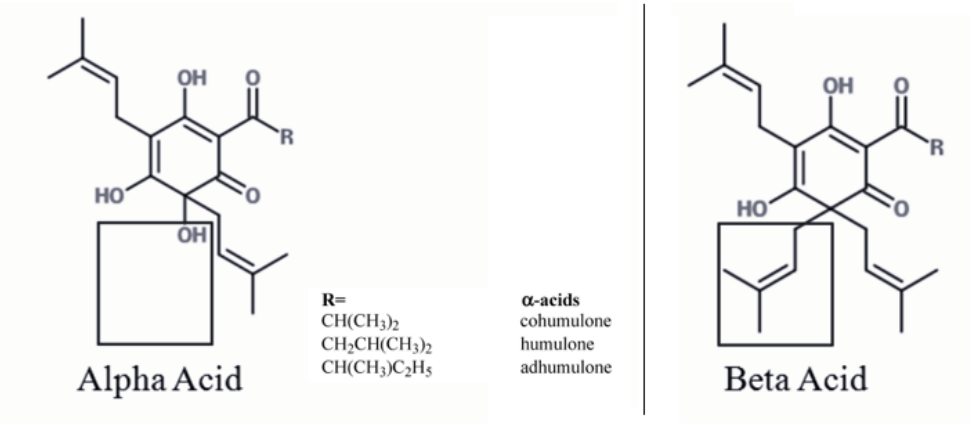
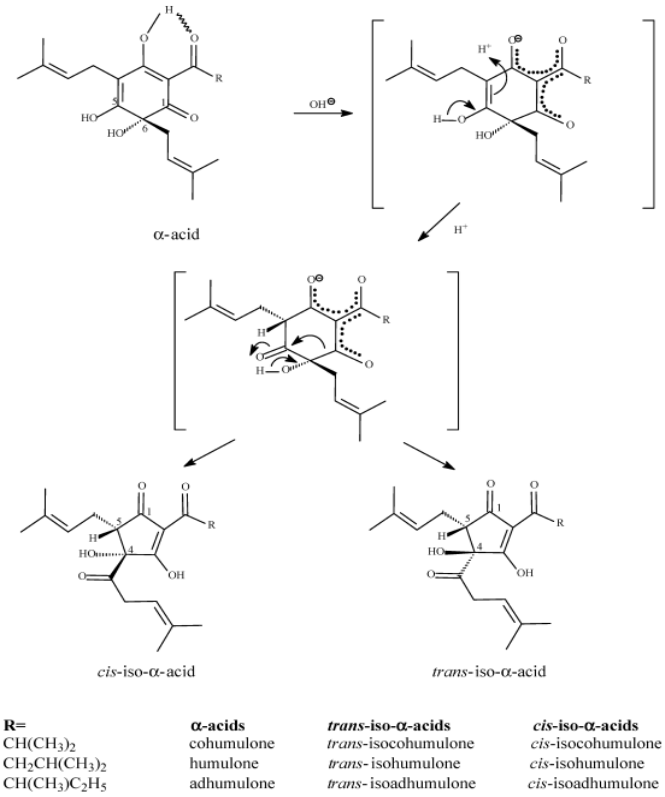


Figure 5 - Chemical formula of alpha acids and beta acids (Goiris et al., 2010) adapted.

The main contribution of hops to the beer production process are the α-acids which are isomerized into iso- α -acids and contribute to the bitter taste on the final beer. These isomerized compounds are much more soluble in water than them precedents, and it is also known that this chemical reaction follows a first-order reaction. (Jaskula et al., 2008).

There are three main homologs of α -acids: humulone, co-humulone, and ad-humulone (depending on the group "R" from the chemical formula of the Figure 5. It depends on the variety of the hop, but normally, the portions of α-acids stand by 10 - 15 % ad-humulone, 20 to 65 % co-humulone, and 35 to 70 % n-humulone (Kostrzewa et al., 2016). One of the homologs, co-humulone, has more negative role in beer bittering (Kunze, 2004a).

These three homologs make, according to Kappler et al. (2010) around 90 % of the α -acids, with some studies (Huang et al., 2013) appointing to 98 % of the total of α -acids. The mechanism for the transformation of α-acids into iso- α-acids is represented in the Figure 6 below.



R=	α-acids	*trans*-iso-α-acids	*cis*-iso-α-acids
$CH(CH_3)_2$	cohumulone	*trans*-isocohumulone	*cis*-isocohumulone
$CH_2CH(CH_3)_2$	humulone	*trans*- isohumulone	*cis*-isohumulone
$CH(CH_3)C_2H_5$	adhumulone	*trans*- isoadhumulone	*cis*-isoadhumulone

Figure 6 - Process of the isomerization of alpha acids into iso-alpha acids (Goiris, et al., 2010).

The transformation of α-acids into iso- α-acids occurs during wort boiling. During this part of the process α-acids isomerize into iso- α-acids via acyloin-type ring contraction (Figure 7). Each iso- α -acid is capable to rise two epimeric iso- α -acids, depending on the spatial arrangement of the tertiary alcohol function at C4 and the prenyl side chain at C5 (Figure 6): cis-iso-α-acids and trans-iso- α-acids (Caballero et al., 2012).

The terms *trans* and *cis* means that these groups point, respectively, to opposite faces and to the same face of the five-membered ring as in the previous Figure 6. This conversion results in six iso- α-acids: the *trans* and *cis* isomers of iso-cohumulone, iso-humulone, and iso-adhumulone (Caballero et al., 2012).

These two stereoisomers, *cis* and *trans,* differ in their properties. It is known, for example, that *cis*-iso-humulone is more stable against ageing than *trans*-iso-humulone but has weaker foam stabilising properties. It is also known that combined result in more bitter beers than *trans*-iso-humulones alone, being the *cis*-iso-humulones the bitter ones. Also, during fermentation, losses were shown to be higher in *trans*-iso-humulones (Kappler et al. 2010).

The rate of the transformation of alpha acids into iso-alpha acids is called isomerization. It is important to not misunderstand utilization with isomerization. The first one considers not only the number of alphas

isomerized but also the losses happening throughout the process. Normally, only a third of the total alpha acids appear at the end in the form of their isomers (Jaskula et al., 2008).

Isomerization only indicates the conversion of alpha acids detected in the medium into iso-alpha acids. (Malowicki & Shellhammer, 2006) The isomerization rate is given by equation 1.

$$Isomerization\ (\%) = \frac{Iso{-}alphas\ detected\ in\ the\ final\ beer}{Alphas\ detected\ in\ the\ medium\ at\ the\ begining} \times 100 \quad (1)$$

The isomerization is not a very efficient process, with conventional hops, normally a maximum of 50-60 % of α-acids being isomerized in traditional brewing and no more than 25 % of the bittering potential going through to the final beer (Caballero et al., 2012).

HOPS OILS

Hops contain more than 300 substances (essential oils) which are volatile on boiling. During this part of beer production, hop oils increasingly evaporate, and in order to preserve some of the aromatic hop oil, a part of it is added later to beers sometimes (Kunze, 2004a). Aroma hops are weaker in α-acids and richer in essential oils. In contrast, bitter hops have a more α-acid content and less essential oils (Brányik & Pires, 2015).

POLYPHENOLS

Polyphenols consist of a mixture of tannins, flavonols, catechins and anthocyanogens, and have important properties for brewing. They have an astringent taste and combined with complex proteins and iron salts form black compounds. As a result, these substances contribute to the final taste and colour of beer. Besides that, hops also have in its constitution proteins and minerals (Kunze, 2004a).

2.2. Beer Production

The process of beer production basically consists of the transformation of the raw materials into fermented and maturated beer. This happens with the assistance of the action of enzymes, which convert

the polymeric macromolecules present in malt (such as proteins and polysaccharides) into simple sugars and amino acids, making them available for the yeast during fermentation. However, there are also some stages that precedes and proceeds fermentation and have equal importance for beer production (Bránayik & Pires, 2015).

It is possible to split the beer process into three phases. Figure 7 represents all of them, where it is possible to verify the three main stages: malting, wort production and fermentation and finishing processes

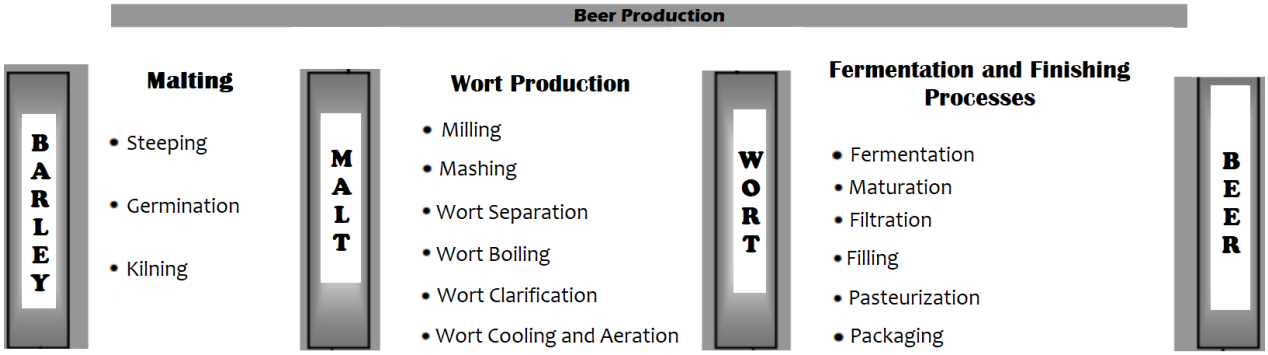


Figure 7 - The beer production (Universiade do Minho, 2020) adapted.

2.2.1. Malting

Barley malt is the main raw material and the main starch source for brewing worldwide. Before being use in the process, barley must be transformed in malt. There are mainly three important steps during malting: steeping, germination and kilning. (Kreisz, 2009)

The malting process is represented in Figure 8 below.

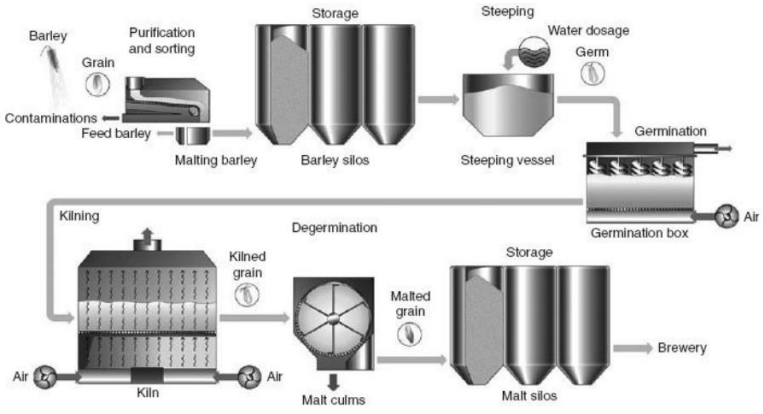


Figure 8 – Steps of malting. Wunderlich & Back, 2009) adapted.

Before anything else, barley goes through a cleaning process in order to avoid contaminations and is dried and stored for further use. The storage happens in silos with aeration, drying and cooling facilities, and temperature and pest control (Kreisz, 2009).

Then, there is the steeping step, in which the main purpose of it is to increase the moisture content of the grain (43 – 46 %), because kernels do not germinate with low moisture content. Besides that, it is also important to wash the grain and remove some germination inhibitors (Kreisz, 2009).

Firstly, a dosage of water is added, in steep vessels, to increase barley moisture following an air rest. After that, a second and often also a third water stage happens. These stages of the process are important to supply barley with water and oxygen (Bamforth, 2003).

Then, germination follows. This is a physiological process where the embryo develops rootlets and acrospires. During this step, growth processes occur, and enzyme formation and metabolic changes happen, which are the main purpose of germination (Kunze, 2004b).

Germination boxes are used for this process. Generally, it occurs in a pneumatic plant at 16 - 20 ˚C that generates great amounts of heat. During this phase the formation of enzymes happen, amylases inclusive, which are important for the mashing process (Bamforth, 2003).

Finally, malt is dried by kilning. This process aims to end the modification and the growth of the plant; to reduce moisture levels in the way that is suitable for grain storage; to conserve the enzyme complexes developed before this stage and develop colour and flavour of malt. During this stage, enzymes present on malt cannot be destroyed, since they have an important role for brewer needs (Bamforth, 2003).

For this part of the process, malt is transferred to kilns which have a false bottom that enables hot air to pass through the green malt (malt after germination) and dry it. Normally, to dry the malt, the temperatures start at around 50 ° C to 65 ° C, in order to promote a significant decrease of malt moisture. The temperature is slowly increased for around 18h, until achieving numbers of around 75 ˚C to 110 ˚C, depending on the type of malt required. This allows obtaining a malt moisture content of less than 4 % at the end. Then, malts should be cooled, cleaned and stored in silos before its use in breweries (Bamforth, 2003 ; Kreisz, 2009)

2.2.2. Wort Production

To obtain beer, one of the most important processes is wort production for its subsequent fermentation. This step has a hugely important role since it is the one responsible to provide fermentable

sugars and important hop compounds in wort (Kunze, 2004c). Figure 9 represents all the stages of wort production.

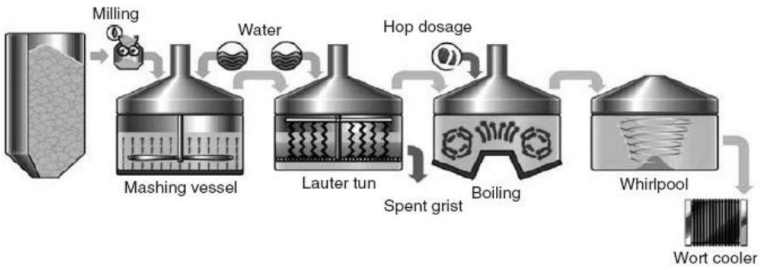


Figure 9 – Steps of wort production (Wunderlich & Back,2009) adapted.

2.2.2.1. Milling

Milling reduces the particles of malt into smaller ones and enables the starchy endosperm to become better accessible to malt enzymes. During milling, the husks must be treated carefully, since they are used as a filter during lautering, a further step in wort production. The fragmentation of malt should be done considering the methods of mashing and separation that are being used.

Malt is fragmented in a grist mill and depending on the process it could be made through hammer milling, wet milling and dry milling (the most common one) (Willaert, 2007).

2.2.2.2. Mashing

The main purpose of mashing is to mix malt grist, solid adjuncts and hot water together at a certain temperature for the malt enzymes to convert different components from the cereal, mainly starch, turning them into fermentable sugars and other nutrients (Leiper & Miedl, 2006). From the technological point of view, mashing can be carried out by either infusion or decoction process.

Starch degradation happens in essentially three phases: gelatinisation, liquefaction and saccharification. Firstly, gelatinisation occurs at approximately 60 - 65 ˚C in barley starch, with the formation of a hot aqueous and viscous solution which is provided by the starch content. (Willaert, 2007)

Then, liquefaction proceeds. Here, the long chains of glucose start to break into smaller chains by the action of α-amylase, once the temperature window in wort is very close to the optimum conditions for this enzyme to act. By liquefaction is meant the reduction of viscosity of gelatinised starch by α-amylase (Kunze, 2004c).

Saccharification is a major step at this point because it is responsible for the formation of smaller sugar molecules in the medium. The two main enzymes that act in this process are α-amylase and β-amylase. The first one breaks down long starch chains into smaller dextrins and acts optimally at 72 – 75 ˚C with a pH of 5.6 to 5.8. Enzyme β-amylase, on the other hand, splits maltose from the non-reducing ends of chains and produces maltotriose and glucose, with optimum temperatures at 62 to 65 ˚C with a pH of 5.4. Both have optimum window temperatures in which they can enhance their work in the medium (Kunze, 2004c).

The acting period of these enzymes can depend on the type of beer that is being made since β-amylase breaks the chains into highly fermentable sugars and α-amylase not so much, contributing more to the beer body. To inactivate these enzymes a mash out must be performed in order to fix the amount of fermentable sugars (Kunze, 2004c).

2.2.2.3. Wort separation

At this part of the process there are essentially two main portions constituting the mash: the undissolved substances called spent grains and the (sweet) wort which is going to be used for beer production. Then, wort separation (lautering) occurs, and this step is used to recover as most of the extract as possible. Lautering is a filtration process in which the spent grains play the role of the filter material. It happens in two stages: the running off the first wort and the sparging or wash out (second wort) (Willaert, 2007).

The running off the first wort consists of draining all the wort from the insoluble parts present in it to a *lauter tun*. After that, sparging happens in which the remaining grains are showered in order to retain some more content from them and transferred to the *lauter tun*. Sparging gradually dilutes wort, and so, the first wort must contain 4 to 6 % more extract than the beer to be produced (Kunze, 2004c).
After the last runnings have run off, all the spent grains are removed from the system, and the wort goes to the boiling equipment for the wort boiling to happen (Kunze, 2004c).

2.2.2.4. Wort boiling

In wort boiling hops are added to (sweet) wort obtained by lautering. Some changes occur in it, of which stands out the extraction of hop compounds, the evaporation of water and undesirable aroma substances and the destruction of all enzymes existing in the wort. (Krottenthaler et al., 2009)

Boiling is the most energy-consuming part of the brewhouse process, it requires a lot of energy since it is performed under high temperatures (Leiper, 2006). Normally, the obtained wort is boiled for 60 to 90 min, depending on the characteristics of the final beer (more time, more evaporation, less wort for the fermentation process), at around 100 ˚C (atmospheric conditions) in the wort kettle. Nonetheless, every boiling temperature (Appendix A.1) is associated with a certain steam pressure, and the higher this pressure is, the higher will be the temperature associated with it (Kunze, 2004c).

The extraction of hop compounds, in particular α-acids, is a step with great importance and influence in the process due to is bitter taste given to the final beer. Boiling changes the structure of the α-acids, isomerising them into iso-α-acids as seen before in topic 2.2.4.1. (Leiper, 2006).

The isomerized compounds are more soluble than the compounds from which they were formed. The efficiency of the isomerization is not very high, though, since only about a third of α-acids are recovered in wort boiling in the isomerized form, and some of them are removed during the rest of the processes. The isomerization depends on many factors: the nature of iso-α-acids, the duration of boiling, the pH (higher pH, higher isomerization) and concentration of α-acids are some of them (Kunze, 2004c).

Hop oils and hop polyphenols also suffer changes during boiling. Hops oils are volatile during wort boiling and hop polyphenols are water soluble and dissolve immediately in the medium. At the end of boiling, the wort becomes more acidic since the compounds formed are acidic and the hops also have an acidic impact in wort (Kunze, 2004c).

2.2.2.5. Wort clarification

After boiling, hot trub and hop debris are removed in order to secure the stability of beer and to prevent the blocking of the plate heat exchanger during cooling. Hot trub particles give beer a darker colour and a poor foam stability. Generally, the separation of hot trub and hop debris happens in a *whirlpool,* promoting the separation of the clear liquid of interest, accumulating the trub particles in the centre of the tank. The circular movement generates a centrifugal force, and the solids are directed towards the edges of the tank. The clarified wort can be recovered slowly by the bottom of the edges of the tank (Leiper & Miedl, 2006).

2.2.2.6. Wort cooling and aeration

The yeasts can only act during fermentation at low temperatures and so, the wort must be cooled as quickly as possible to 5 ˚C to 6 ˚C, or often to 7 ˚C to 10 ˚C. During this process more solids may

precipitate (cold trub), these solids are essentially proteins and lipids and could be removed by flocculation, flotation, centrifugation and/or filtration (Kunze, 2004c). Customarily, cooling is achieved using plate heat exchangers. Besides that, yeasts also need oxygen to multiply, and the wort must be aerated. For the yeast propagation it is recommendable a target of 70 % to 90 % saturation of oxygen in wort which gives a content of 4-14 mg/L of it in the fermentation vessel. (Leiper & Miedl, 2006)

2.2.3. Fermentation and finishing processes

The transformation of wort into beer is the third main step in brewing. The main step of this part of the process is fermentation, which metabolizes sugars (produced in the wort production phase) into ethanol and CO_2 (products), by the activity of microorganisms, gaining energy in the process.

Figure 10 assembles the third and final part of the brewing process.

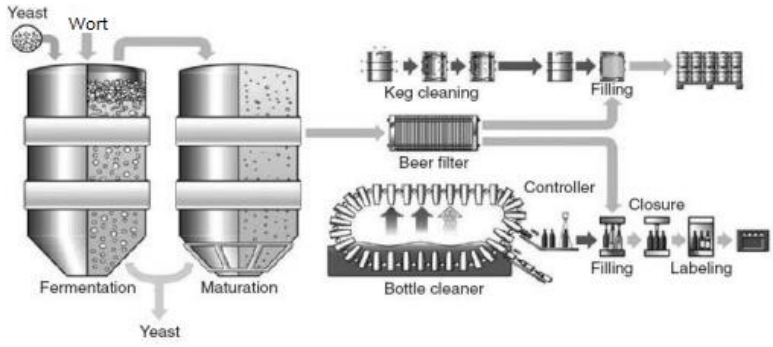


Figure 10 – Steps of fermentation and finishing processes (Wunderlich & Back,2009) adapted.

2.2.3.1. Fermentation

Fermentation takes around 7 days and is characterised by the fermentation of the wort by free cells in suspension inside fermentation vessels. On often used type are *cylindroconical vessels.* (Munroe, 2006).

The process is initiated by the addition of yeast ("Pitching") from the genus *Saccharomyces* into the wort, and after this moment the wort is called by the name of "green beer" or "young beer". The fermentation, depending on the type of yeast used, can be considered a top or bottom fermentation (Eßlinger, 2009).

Top fermentations, also called *Ale* fermentations, make use of the yeast *Saccharomyces cerevisiae*, a top-cropping yeast incubated at 15 °C to 20 °C. This strain of yeast rises to the top of the beer toward

the end of fermentation (floating yeast). When beers are made through this process, they are called *Ale* beers (Munroe, 2006).

In the other hand, bottom fermentations or *Lager fermentations,* use *S. pastorianus* (previously classified as *S. carlsbergensis*) yeast species. In this case yeasts form flocs or clumps, which being denser than beer, tend to settle to the bottom of the tank towards the end of fermentation. The process is executed at considerably lower temperatures when compared with *Ale* beers, with temperatures between 6 °C to 14 °C, making it a longer process. This process results in *Lager* beers. (Munroe, 2006).

The yeast consumes all the oxygen and after a few hours CO_2 bubbles, foam and heat are created. Subsequently the yeast growth and sugar consumption rates reach a maximum. The pH decreases (3.8 - 4.4) due to the production of organic acids and the consumption of buffering compounds with a slightly increase towards the end, due to the reduction of the growth rate. Low pH values inhibit bacterial growth and degradation during fermentation (Munroe, 2006).

During fermentation, yeast will act in fermentable sugars in the wort, producing ethanol. The yeast first hydrolyses glucose and fructose (primary sugars), then degrade maltose and maltotriose (secondary sugars). There are also some non-fermentable sugars which is the case of dextrins. Fermentation ends when all the fermentable sugars are consumed (Munroe, 2006)

Besides CO_2 and ethanol, fermentation produces other compounds which contribute to the sensory properties of beer such as diacetyl, higher alcohols and esters. (Munroe, 2006)

2.2.3.2. Maturation

Maturation, also called second fermentation, is performed in order to remove undesired substances in beer, in particular diacetyl, which gives bad flavours to the final beer and its values should be reduced (< 0.1 mg/L). This process can be executed in the same vessels of the fermentation process or in another vessel for maturation (Brányik & Pires, 2015).

Traditionally, maturation involves a secondary fermentation that is affected by the small amount of yeast and fermentable sugars remaining in the beer which forms CO_2. When the levels of diacetyl are achieved, the temperature of beer is decreased by 1 °C for a certain period of time, in order to clarify and stabilize beer. Low temperatures are practised since low temperature induces a quicker sedimentation. Finally, after its stabilization, the beer is ready to proceed into final processing stages (Brányik & Pires, 2015; Kunze, 2004d).

2.2.3.3. Filtration

At the end of the maturation process, beer is oxygen-free, although still has turbidity-causing particles. Then, filtration happens which is a separation process in which all the turbidity- causing constituents in beer are removed. The main purpose of this step is to preserve beer so that no visible changes arise for a long time (Kunze, 2004d).

The beer is separated by a filter into a clear filtrate and a filter cake which is left behind. At the end of filtration, all the suspended materials and potential turbidity former are removed, resulting in a clarified beer (Lindemann, 2009).

2.2.3.4. Filling

After filtration, beer is ready to go to the filling process. Nowadays, beer is filled mainly into glass bottles, but there are also other ways to do it such as: plastic bottles, cans, kegs or smaller containers (Kunze, 2004e).

For a success filling of beer, it is necessary to take into account a series of parameters in order to avoid the access of air to beer. Breweries tend to have proper machinery to execute the filling process of bottles, that enables to reduce product loss, filling with consistent contents, no microbiological contamination and no loss of CO_2/appearance of oxygen during the process. Normally, the filling is complemented by the labelling of the bottles (Lindemann, 2009).

2.2.3.5. Pasteurization

In order to secure the shelf life of the beer, pasteurization is the best method, especially if there are still some fermentable sugars in it. This is not a practice adopted by all breweries, in fact, craft beers tend to avoid pasteurization or other sterilization method. When it occurs, it is performed in tunnel of flash pasteurizers. (Dunn, 2006)

2.2.3.6. Packaging

For sale and to facilitate the manipulation with small transport packages, they are packed and transported in packaging units. The packaging must have light-proofing characteristics and ability to withstand mechanical stresses and breakage.

Usually, these packaging units are crates, cartons, trays or six packs and are stored in pellets before the transport to the final destination (Kunze, 2004e).

2.3. Cavitation and hydrodynamic cavitation

Cavitation, in general, can be defined as the phenomenon of formation, growth and rapid collapse of bubbles (cavities) in a liquid, generating significant amounts of heat in a controlled system. Contrary to boiling, these vapor structures formation, takes place at a constant temperature, and their appearance is linked to a local drop in pressure generated in the flow rate. (Franc, 2007).

As it is possible to observe in Figure 11, there are mainly four types of cavitation: hydrodynamic, acoustic, optic and particle cavitation.

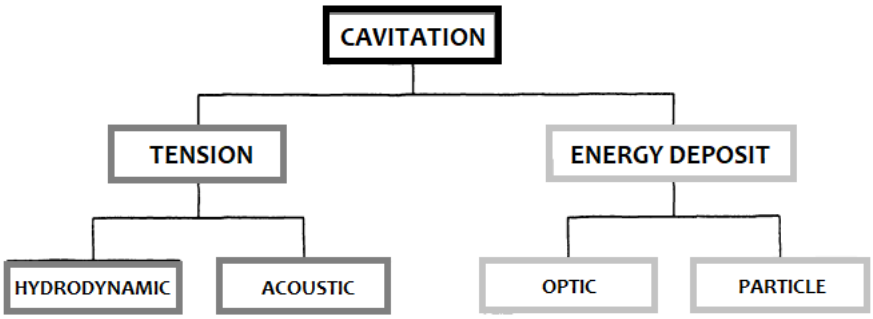


Figure 11 - Types of cavitation.

Hydrodynamic and acoustic cavitation are the two main technologies of interest for academic and industrial use due to the simplicity of operation. These two types of cavitation are the result of tensions prevailing in a liquid (Shah et al., 1999).

In hydrodynamic cavitation, the cavitation is produced by pressure variation in a flowing liquid which is obtained through the geometry of the system, causing velocity variation. In acoustic cavitation, on the other hand, the pressure variations in the liquid are achieved using sound waves (ultrasound) in certain frequencies (16 kHz–100 MHz) (Gogate et al., 2006).

Optic and acoustic cavitations are created because of local energy deposits. These forms of cavitation, though, seem to be more expensive for large-scale operations. The optic cavitation is produced via laser, bursting the liquid. Particle cavitation, as the name says, is produced by an elementary particle (neutrons, for example), rupturing the liquid (Shah et al., 1999).

2.3.1. Principles of hydrodynamic cavitation

For hydrodynamic cavitation (HC) to occur there must be a drop in pressure in the circulating liquid. This pressure drop can be achieved with the help of a pump, to promote the circulation of the liquid, and a hydrodynamic cavitation reactor (HC reactor) which could be an orifice plate or a Venturi tube, for example (Carpenter et al., 2016).

The flowing stream enters the HC reactor, and a high pressure is generated, due to the constriction in the flow. At this constriction, the velocity of the stream increases, causing a drop in the pressure, below the saturated vapour pressure of the liquid. At this moment, the conditions are reunited to form bubbles (cavities) in the stream (Michel & Kozyuk, 2015).

The bubbles formed go with high velocities to the zone after the constriction, which is under hydrostatic pressure because the full recovery pressure does not take place. This low pressure induces a violent collapse of the cavitation bubbles, which is a result of the energy accumulated during the growth processes of the bubbles and it is transferred to the liquid flow (Michel & Kozyuk, 2015).

The process is represented schematically in Figure 12.

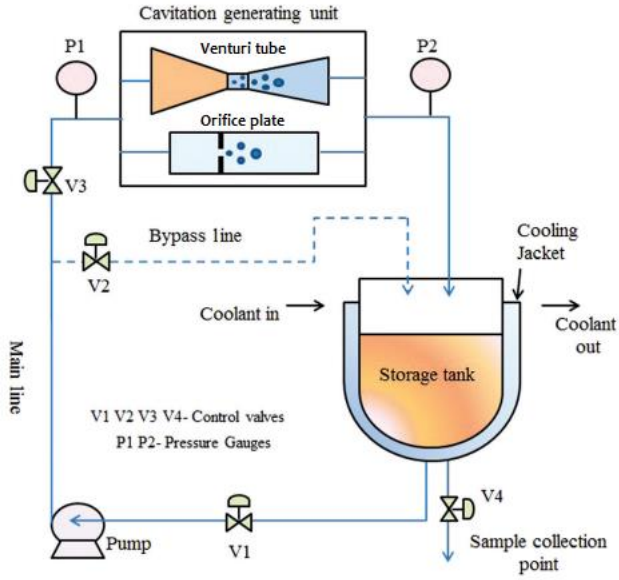


Figure 12 - Scheme of the hydrodynamic cavitation method (Carpenter et al., 2016).

The next Figure 13 shows the formation, growth and collapse of the bubbles generated by the HC reactor.

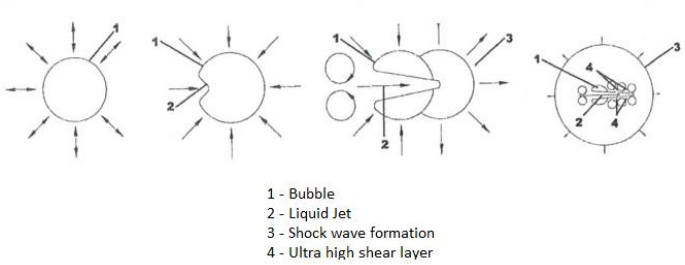


Figure 13 - Formation and collapse of the bubbles during hydrodynamic cavitation (Michel & Kozyuk, 2015) adapted.

During the process represented in Figure 13 some important chemical transformations take place. These chemical transformations happen essentially via two mechanisms: primarily though the formation of reactive free radicals of the substrate molecules, and secondarily through thermal breakdown of larger molecules into smaller molecules under the conditions of a local hot spot (Carpenter et al., 2016).

In the core of the cavities (bubbles), the temperature and the pressure reach high levels (10 000 K and 1000 atm, respectively) during their collapse. Inside this core (hot spot), the molecules that were trapped dissociate into smaller ones, producing reactive free radicals upon dissociation. These radicals act on the molecules, dissociating them into OH and H radicals. These hydroxyl radicals have a very high oxidation potential, which can oxidize large organic molecules (Carpenter et al., 2016).

At the interface of the bubbles, microjets and turbulence (point 2 shown in Figure 13) are created due to the cavity oscillation and consequent collapse. In that region the temperature may reach 2000 K. The oscillation and turbulence boost the transport of the generated radicals which react with organic molecules. Also, due to the high temperatures in this region, the organic molecules get thermally decomposed (Carpenter et al., 2016).

In the bulk liquid region, farthest to the core, the temperature is not so high and pressure remains near the atmospheric temperature. In this region, the generated radicals diffuse into the bulk liquid and react with the targeted molecules (Carpenter et al., 2016).

The HC is influenced by diverse factors such as the HC reactor geometry, the flow rate and the inlet pressure, the geometry and the mechanical power of the impellers, and also, the content in dissolved gases and solid particles and the temperature of the medium (Meneguzzo et al., 2019).

To generate the hydrodynamic cavitation, liquid is pumped through the devices referred before as HC reactors using a pump. The pump rating is decided by the cavitation number and volumetric flow rate as required for a particular application. These terms will be covered in the following topics (Carpenter et al., 2016).

2.3.2. Cavitation number

The cavitation number (CN) is the main parameter in order to define the intensity of cavitation flow. To understand the origin of the formula of CN, it is necessary to understand the principle of Bernoulli of which it derivates (Meneguzzo et al., 2019).

The Bernoulli´s equation see below (Equation 2), represents the conservation of the mechanical energy for a moving fluid. The liquid acceleration, and the respective pressure drop, described by it.

$$P1 + \frac{1}{2}\rho v_1^2 + \rho g h_1 = P2 + \frac{1}{2}\rho v_2^2 + \rho g h_2 \quad (2)$$

In the previous equation, *P1* and *P2* (Nm^{-2}) are the upstream bulk pressure, and the pressure at the HC reactor, respectively, ρ (kgm-3) is the fluid density, v1 and v2 (ms-1) are the fluid speed upstream and through the HC reactor, in that order. The *h1* and *h2* (m) are the heights of the fluid, and *g* (ms^{-2}) is gravity accelaration. The third term at each side of the equation is the specific potential energy, while the second term characterizes the specific kinetic energy. Assuming equal heights, the pressure drops after the HC reactor (*P2<P1*), because of the fluid acceleration due to mass conservation (*v2>v1*). Whenever *P2* drops below the vapor pressure, at a specific temperature level, local evaporation occurs, and vapor bubbles are produced (Meneguzzo et al., 2019).

As it was referred before, the CN is derived from Bernoulli´s equation. It is a dimensionless number and represents the hydraulic characteristics of the cavitation device and the tendency of the flow to cavitate (Terentiev et al., 2011). The deduction of the CN formula from the Bernoulli´s equation is represented below (Equation 3).

$$CN = \frac{P2 - P_v}{\frac{1}{2}\rho V_0^2} \quad (3)$$

From the previous equation, where *Pv* is the liquid vapor pressure and, *P2*, the pressure after the constriction. The other symbols were already referred in Equation 2. The CN represents the ratio between the pressure drop needed to achieve vaporization (represented in Figure 17), and the specific kinetic energy at the cavitation inception section. When the inlet pressure is increased, the flow rate of the system also increases (velocity increases), makes the cavitation number decrease (Nilesh et al., 2000).

In order to understand a bit better the formula, in Figure 14 is represented what happens in terms of pressure in a HC reactor. The numerator from the cavitation number equation is given by the difference between *P2* (pressure out of HC reactor) and *Pv*, the vapor pressure of the liquid (Meneguzzo et al., 2019).

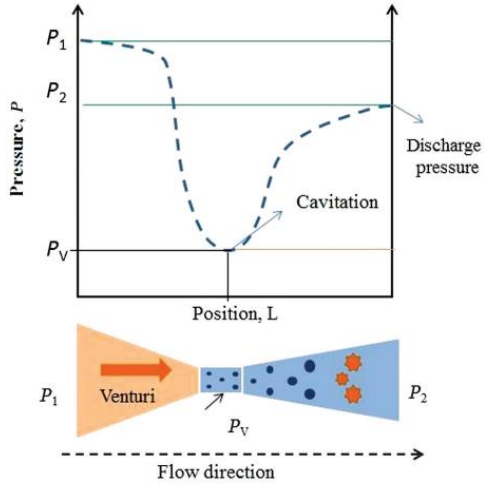


Figure 14 - Pressure variation in the hydrodynamic cavitation reactor (Carpenter et al., 2016).

The cavitation number can be defined in a certain range of values. At low cavitation numbers (CN<1), higher cavitation yields are formed for most applications, however if it goes beyond a certain value which provokes choked cavitation or super cavitation resulting in vapor cloud formation and no collapse of cavitation bubbles. In the range, CN>1 cavitation becomes more and more residual, depending on the nature and concentration of impurities and dissolved gases. (Albanese & Meneguzzo, 2019).

Depending, on the medium, the ideal cavitation number applied can change. For some fluids an optimum cavitation yield is achieved when the cavitation number is as low as possible (without super cavitation), generating a big number of bubbles and the collapse of them. According to Nilesh et al. (2000) a decrease in the cavitation number results in an increase of the number of cavities getting generated and collapsing per unit time increases.

For slurry and viscous fluids, the cavitation number applied should be higher, and in some situations a cavitation number CN>1 is ideal (Carpenter et al., 2016).

2.3.3. Hydrodynamic cavitation reactor

In order to achieve the cavitation phenomenon, it is very important the presence of a hydrodynamic cavitation reactor, as it was said before, the liquid passing through this reactor produces the cavitation

bubbles. The HC reactor is designed to initiate the cavitation events in a controlled environment, utilizing energy generated by imploding cavitation bubbles, promoting chemical reactions. There are two main types of reactors: Venturi tubes and orifice plates (Zheng, 2021).

Orifice plates can be used with single or multiple holes. When used with multiples holes, they can be with different combinations of diameter of holes and the number of them. This allows obtaining different cavitation intensities (Thanekar & Gogate, 2018).

The most conventional process uses the Venturi tube as a HC reactor. Normally, this reactor consists in three sections: convergent, throat and divergent (Figure 15).

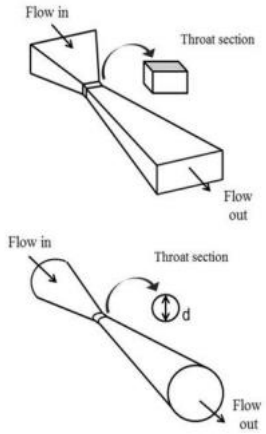


Figure 15 –Venturi tube (Zheng, 2021).

Unlike the orifice plates, in the Venturi tube, the fluid inside of it contracts and expands and the pressure and velocity vary. This gradual change in fluid condition avoids dramatic change in pressure at constriction, which is beneficial for the generation of microbubbles. In the case of Venturi, a relatively higher velocity at the throat is produced as compared to an orifice for a given pressure drop across the device due to its smooth converging and diverging sections (Zheng, 2021).

Both orifice plates and Venturi tubes geometry affect considerably the fluid. In order to achieve a perfect cavitation yield, a series of parameters are important to consider such as: number of cavities generated, residence time of the cavities in the low-pressure region, pressure recovery rate in downstream section, cavity trajectory, and cavitation intensity are very important. All these properties can be regulated varying the geometry of the cavitation device (Carpenter et al., 2016).

The Venturi tube is capable to acquire higher degradation than a orifice tube, being capable to generate more cavities which is associated with more degradation of the particles in a medium (Carpenter et al., 2016).

2.3.4. Pump performance

To generate the cavitation, liquid is pumped through the HC reactor using a pump. The pump rating is decided by the cavitation number and volumetric flow rate as required for a particular application. (Carpenter et al., 2016). In the case of big amounts of fluid with likely solids in suspension, centrifugal pumps are the best type of pump to be used in this case (Universidade do Minho, 2019). Figure 16 represents a characteristic centrifugal pump.

Figure 16 - Centrifugal pump (Franklin Electric, 2022).

To select the right pump, it is important to understand two concepts: the system curve and the pump curve. The system curve defines the head loss resulting from frictional effects (valves, junctions, elbows) as fluid is forced through downstream pipework (green curve in Figure 17). The pump curve, in the other hand, defines the fluid flow and the head for the pump itself (blue curve in Figure 17). When there is no head to work against, the pump achieves a maximum output allowed by its design (Michael Smith engineers Ltd, 2022).

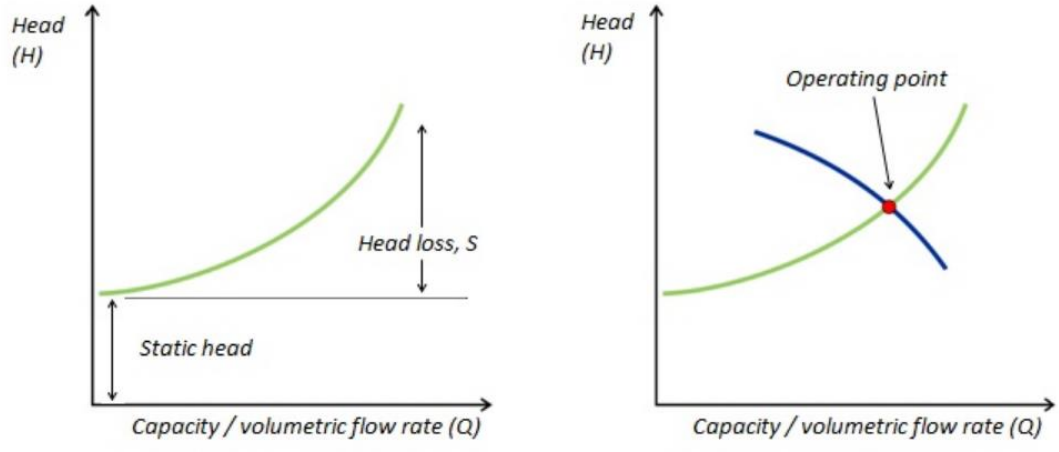


Figure 17 - Pump curve and its operating point (Michael Smith engineers Ltd, 2022).

From Figure 17, the point on which both curves combine conserves the operating point of the system. If the point of execution is too far left of the supposed situation a pump's throughput is lower than its design specification and the fluid may not flow correctly through the system. This could lead to vibration and seal wear. In the case the execution point is too far to the right of the curve the pump's throughput is higher than its design specification and there is a danger of cavitation in the impeller (Michael Smith engineers Ltd, 2022).

2.3.5. Applications of hydrodynamic cavitation

The use of hydrodynamic cavitation has the potential to substitute conventional processes in industry, being more efficient with lower costs. There are a series of sectors where cavitation is being used recently. One of them is the chemical and process intensification in areas such as: oxidation reactions, nano emulsions, hydrolysis of oils, depolymerization reaction, crude oil upgradation and synthesis of biodiesel, due to the capacity of cavitation to work at high temperatures and high pressures (Albanese & Meneguzzo, 2019).

Another sector of great interest for the use of this technology is wastewater treatment. Many industrial, agricultural, medical, and other domestic activities produce wastewater composed of organic and inorganic compounds such as pesticides, drugs and textiles. There are some conventional methods which are used to overcome this pollution but are not capable to mineralize many organic pollutants. Cavitation though, seems to be more efficient and capable to mineralize these pollutants present in wastewater (Carpenter et al., 2016).

Besides that, there are also there some physical processed where the technology seems to be interestingly applied. One of them is the synthesis of nanomaterials, microbial cell disruption and food processing in which this technology is used to replace thermal techniques such as pasteurization (Carpenter et al., 2016).

2.3.6. Hydrodynamic cavitation in beer processing

More recently, the application of hydrodynamic cavitation has been investigated in the brewing sector with some promising results. The technology has been tested in the hot stages of beer processing, in other words, the stages from mixing water with malt to wort heating and hopping (Albanese & Meneguzzo, 2019).

In order to achieve the expected results with the use of the technology, it is necessary to plan the right machinery to execute the cavitation in beer. Maneguzzo and Albanese (2019), patented the technology and commercialize it under the name of CAVIBEER. Basically, it was developed under the microbrewery scale, and it consists of a closed hydraulic loop, powered by a centrifugal pump with the presence of a HC reactor (venturi tube) which is responsible to induce the cavitation process, turning mechanical energy into heat. The scheme used by Maneguzzo and Albanese (2019 is very similar to the one used in this study and represented in section 3.1. (Figure 18).

Studies appoint that the use of this technology could have a major contribution mainly in three sectors: isomerization of alpha acids from hops, the possibility to produce low gluten content beers and energy savings in the process. However, the referred studies do not present many quantitative results regarding those three sectors, and the present study seeks for more results and more accurate conclusions in this topics.

2.3.6.1. Isomerization of alpha-acids from hops

Hydrodynamic cavitation has a high interest in its action on hop constituents. It has the potential to improve the extraction and isomerization of hops alpha-acids into iso-alpha-acids before the onset of boiling, since enhances the expose of the surface of hops and the mass transfer to the wort. The isomerization seems to be possible under the boiling point temperatures, since all the processes could be completed under 100 °C, and the pitching of hops could be done under the temperature that is normally used in conventional methods (not lower than 90°C) (Albanese et al., 2017a).

Besides that, another hop constituents need to be taken into account, such as hop prenylflavonoids which is the case of xanthohumol. These constituents are important to the final properties of beer and its storage stability. It is expected that under certain conditions it is possible to retain higher amounts of xanthohumol when compared with conventional methods (Albanese & Meneguzzo, 2019).

2.3.6.2. Low gluten content

Another topic of interest for the application in brewing is the hypothesis to produce low or free content gluten beers. Beers are produced form cereals, normally from wheat or barley malts, in which there ´s a presence of gluten content. This makes beer unsuitable for consumption by coeliac disease patients. (Hager et al.,2014)

The most important step of brewing production to prevent the gluten content in beers is fermentation. During this phase, the assimilation amino acids occur. Given the fast assimilation of other amino acids such as glutamine, the final gluten content will depend on the assimilation of the amino acid proline. Both amino acids make part of the constitution of the protein gliadin, a component of gluten (Albanese & Meneguzzo, 2019).

The production of very low gluten content beers (<100 mg/L) or gluten-free beers (<20 mg/L) is in its starting phase, and it's expected to be on high level in Europe. Most of the gluten-free beers use a portion of malts derived from cereals and the rest from pseudo-cereals not containing gluten as it is the case of sorghum or quinoa. Although, the brewing methods for cereals different from barley are not very well established. In a way to produce this low gluten content beers, complex and costly methods are applied such as filtration and enzymatic techniques which aim at conditioning the malts in order to boost the processes leading to the precipitation of proteins, in particular polypeptides, during mashing, fermentation and possibly stabilization. Besides that, an alternative is the use of silica gel during fermentation in order to remove proteins, but again it is a costly method (Albanese et al., 2017b).

Apart from the cost of this methods, the final beer falls far away from traditional aroma and flavour. A solution for that, is the use of hydrodynamic cavitation, in which there is evidence that shows the action in conventional barley malts in order to reduce the gluten content in the final beers, without changing any ingredients, using additives or another technology (Albanese et al., 2017b).

Basically, this technology can reduce the gluten content in two steps. Firstly, the proline molecules are exposed due to the shockwaves of cavitation, which are effective to unfold a fraction of glutenin (insoluble) subunits, acting synergically with heat, directly exposing proline molecules. Then, proline

(hydrophobic) tends to locate at the water-vapor interface or inside the cavitation bubbles, where they are destroyed by the collapse and high temperatures of the bubbles. The residues of proline could be assimilated during fermentation and maturation originating gluten free content beers (Albanese et al., 2017b).

2.3.6.3. Energy savings

Beer brewing is very costly when it comes to energy spending, according to Kubule et al. (2016), energy costs in a brewery make up to 9 % of the total production costs. The main processes for this statistic are mashing and wort boiling.

In order to economize energy in the process, many studies suggest the reduction of evaporation rates during brewhouse operations, heat recovery, using thermal energy storage systems and utilizing low temperature heat, minimizing heat losses by improving insulation of process tanks, applying process automation and using variable speed drives) point that improvement of energy efficiency also allows to reduce heat supply capacity and further improve the integration of low temperature heat sources (Kubule et al., 2016).

Hydrodynamic cavitation seems to be a great technology in order to achieve certain savings in beer production. This method allows a dramatic reduction of saccharification temperature (less energy necessary), with an acceleration of the starch extraction. Besides that, it was proven that certain traditional stages are not necessary such as dry milling and wort boiling, which leads to significant time and energy savings, the latter valued around 30 % less (Albanese & Meneguzzo, 2019).

The acceleration of enzymatic saccharification with the technology can be explained by the crushing of particles and disruption of cell walls, with the increase of the surface area. As a result of that saccharification temperatures decrease to values lower than 40 °C, which is, more than 30 °C lower than in traditional brewing processes. During wort boiling, with hydrodynamic cavitation, it is not necessary to increase the temperature to the boiling point. Depends on the cavitation number, but lower temperatures can be used, just making sure that is an enough temperature to remove undesired volatile aromatic compounds such as dimethyl sulfide and guaranteeing the isomerization of alpha acids (Albanese & Meneguzzo, 2019).

According to Kubule et al. (2016), 93 % of the heat losses are due to the evaporation phenomenon that happens during wort boiling. Also, during wort boiling, the specific energy demand is very high (24-

54 MJ/hl), this way, if the temperatures applied during this stage are lower, the energy consumption will also be lower (Slawitsch et al., 2011).

3. MATERIALS AND METHODS

3.1. Brewing Units

In order to investigate the effects of hydrodynamic cavitation in brewing, a layout of equipment was built from available commercial components. Figure 18 shows the experimental scheme of the installation of all the process.

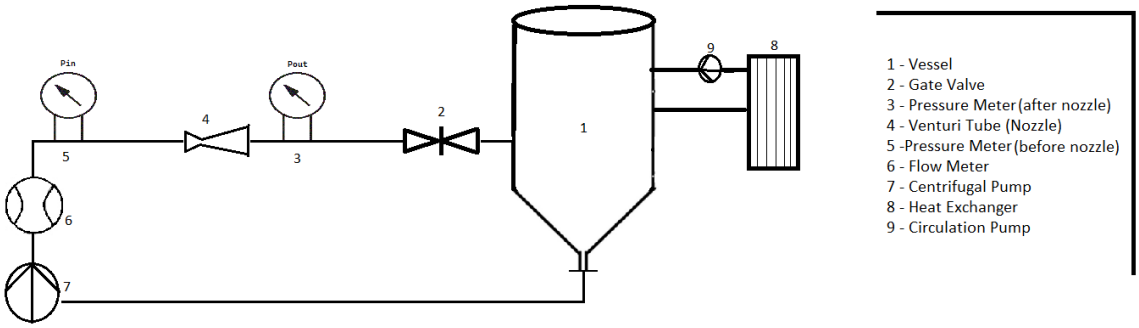


Figure 18 - Experimental scheme of the process.

This installation includes a stainless-steel vessel for the wort boiling with a capacity of 110 L, which was powered by a centrifugal pump (E-Tech EH/08 I015 T5 E0 IE3, Franklin Electric, Czech Republic). The pump, according to the information in the manufacturer's technical documentation, has a nominal mechanical power of 1.5 kW and an input power of 1.39 kW. Besides that, it allows to change the frequency from one phase to a three-phase motor pump. All the parts in contact with liquids are made from stainless steel.

The circulating liquid (wort) was in a hydraulic loop through stainless steel tubes with 25 mm of diameter. The wort was exposed to an atmospheric pressure which could be manipulated after the nozzle (Venturi tube with 2.6 mm of diameter), by a gate valve that controls the flow and consequently the cavitation number in the process. The geometrical characteristics of the Venturi tube used in the experiences are represented in the next Figure 19.

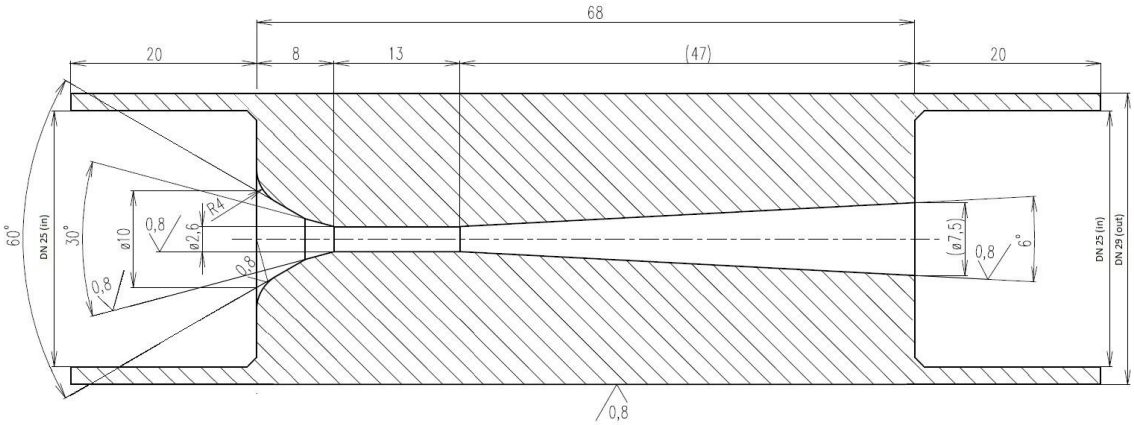


Figure 19 - Geometrical characteristics of the Venturi tube.

When the valve is completely open, the pressure measured (P_{out}), downstream the cavitation reactor, is the atmospheric pressure. The flow rate was also controlled manipulating the frequency of the pump.

The pressure in the system was measured by two pressure meters situated in different places of the tubes: one of them, P_{in}, measures the pressure between the pump and the nozzle, and the other one, P_{out}, measures the pressure out of the nozzle, situated between it and the valve responsible to control the cavitation and flow. Thus, to control the flow, the system was equipped with a flowmeter, also represented in the scheme.

Moreover, a smaller circulation pump led a secondary recirculation loop through a heat exchanger, which allows maintaining a certain temperature during the wort boiling process, depending on which experience is being performed.

The brewery was equipped with automatic valve systems in almost all the spots, through control via a computer program developed by the "Project Soft" company (Czech Republic) which built the program to control all the brewery through the computer. The program assisted all the process, including, the valves and the temperature of the wort boiling via action on the heat exchanger.

3.2. Brewing Ingredients

Hop extract, hop pellets and malt concentrate were used in the performed tests.

The malt concentrate was a Czech product (Sladovna Bruntál, Kanditní sladový výtažek) which is made from Pilsen type malts. The malt concentrate used is represented in Figure 20.

Figure 20 - Malt concentrate used in the experiences.

The hop extract used was produced from a company called "Bralex". It is a CO_2 hop extract obtained from German hop variety Herkules, with 52.96 % of alpha-acids in its constitution. The hop extract used in the experiences is represented in Figure 21.

Figure 21 - Hop extract used in the experiments.

Lastly, hops in pellet form were also used for some experiments. These hop pellets (Premiant 2017, Bralex, Czech Republic) have 7.6 % of α-acids. The hop pellet used in the experiences is represented in Figure 22.

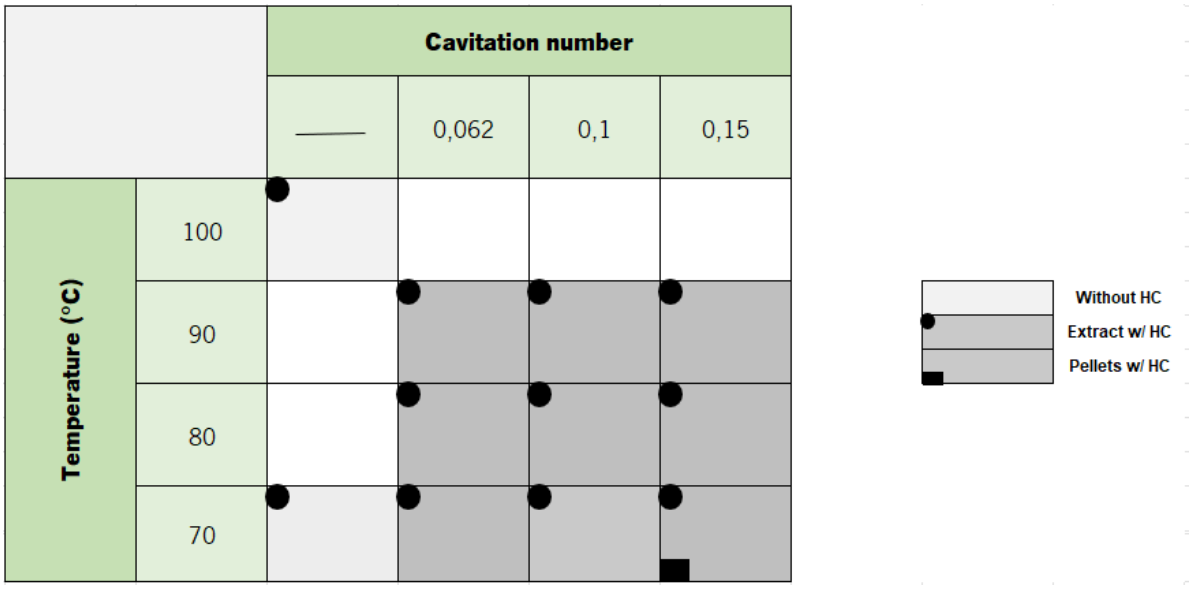
Figure 22 - Hop pellets used in the experiments.

3.3. Planning of the experiments

These experiments aim answering to the main question of the study: on which conditions should the process of wort boiling be performed in order to optimize the isomerisation of alpha-acids.

The Table 1 represented below, shows all the conditions used in the various experiments that were made during the study of HC effects in wort boiling.

Table 1 - Planning of the experiments



The experiments were performed testing different temperatures and cavitation numbers in wort boiling. In some experiments, hop pellets were used instead of hop extract in order to see if its use is possible in the experiments and compare its efficiency with hop extract.

The cavitation number was given by Equation 3 (theorical part). It depends on the flow rate, and on the pressure out of the nozzle. Then, before each experiment it was necessary to manipulate the pressure out of the nozzle and the frequency of the pump in order to achieve a certain flow rate. Moreover, the cavitation number also depends on the vapor pressure (Appendix A.1) which depended on the temperature at which the experiment was performed. Also, it depends on the density that is related to the *Plato* degree of the beer it was being made (Appendix A.2)

3.4. Experiments in practice

The experiments with wort boiling have been done in the technological department of the RIBM Prague. These, were carried always adopting the procedure described on this topic, just making the changes on the parameters described in previous Table 1.

Figure 23 concerns the scheme for assembling the process.

Figure 23 - Process assembly scheme

Firstly, the vessel was filled manually with 50 L of water, followed by heating it up until the desired temperature through the heat exchanger. The activation of the heat exchanger was manipulated by the computer programme a represented-on Figure 24 below. The green path in the figure represents the loop executed in the system. All the valves (automatic and manual ones) must be closed until the filling process is finished, to make sure the water does not leave the system.

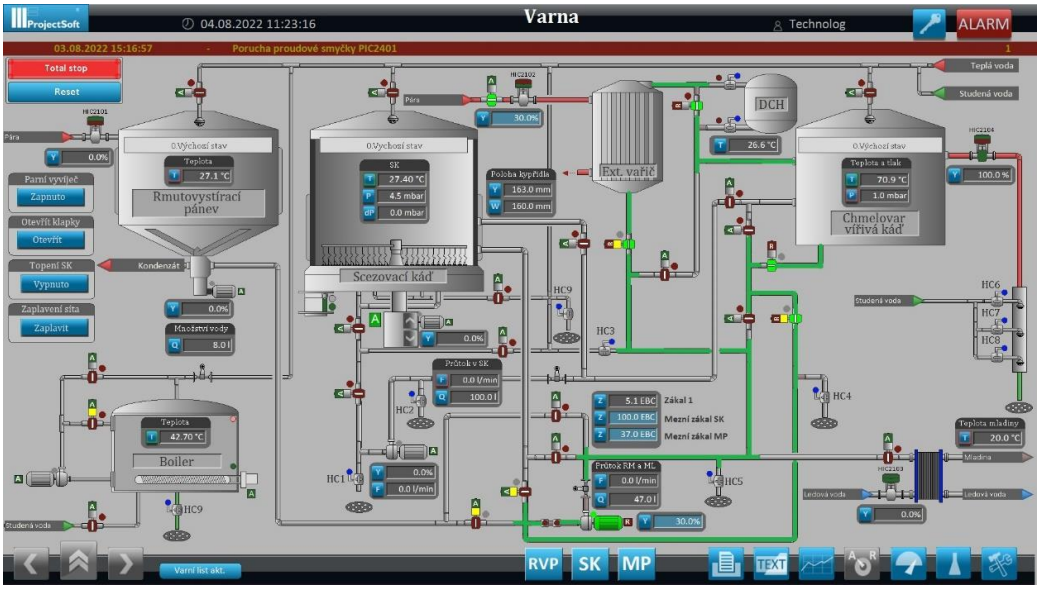


Figure 24 - Activation of circulating path and the heat exchanger in the program "Project Soft".

When the temperature was getting close to the target, it was added malt concentrate "Sladovna Bruntál" (previously weighed on a scale). The amount of malt has been calculated according to Equation 4, where *V*, represents the volume of water in the vessel.

$$m_{malt} = 0.15 \times V \tag{4}$$

When all the malt was transferred to the tank, the medium was stirred to form a homogeneous mixture. As soon as the desired temperature is reached, the heat exchanger was turned off. After that, the pump for cavitation was switched on, and its frequency and the valve after the Venturi tube were both manipulated to achieve the required flow rate (visible on the flowmeter), which will correspond to certain cavitation number.

The required amount of hop was measured on a scale and added the medium (containing 93.22 mg/L alpha acids content), followed by a quick stirring. Equation 5 was used to determine the quantity of hop used.

$$m_{hop} = \frac{IBU \times V}{A[\,\%] \times U[\,\%]} \tag{5}$$

In Equation 5, *V* represents the total volume of wort, *A* the percentage of α–acids in the hops used, *U* represents the efficiency of the brewery and IBU is the bitter potential of hops.

After that, a sample was taken from the system, through a manual valve from the tank, corresponding to the "minute 0", when the experiment itself started. During the next 90 min, samples were taken into falcon tubes every 15 min to make up 7 small samples taken during that time. During the same time four extra samples were taken into small plastic bottles (Figure 25), corresponding to 0, 30, 60 and 90 min, in order to make gluten analyses. As the samples were taken, they were immediately placed in the freezer for further analysis as it is complemented in Figure 25.

Figure 25 - Samples collected in the refrigeration system.

After all the samples were collected, the pump was switched off, and all the valves in the system were opened, both automatic, via the computer program, and the manual ones in order to release all the wort inside the tank. Figure 26 shows the automatic valves that were opened through the menu signalized in it.

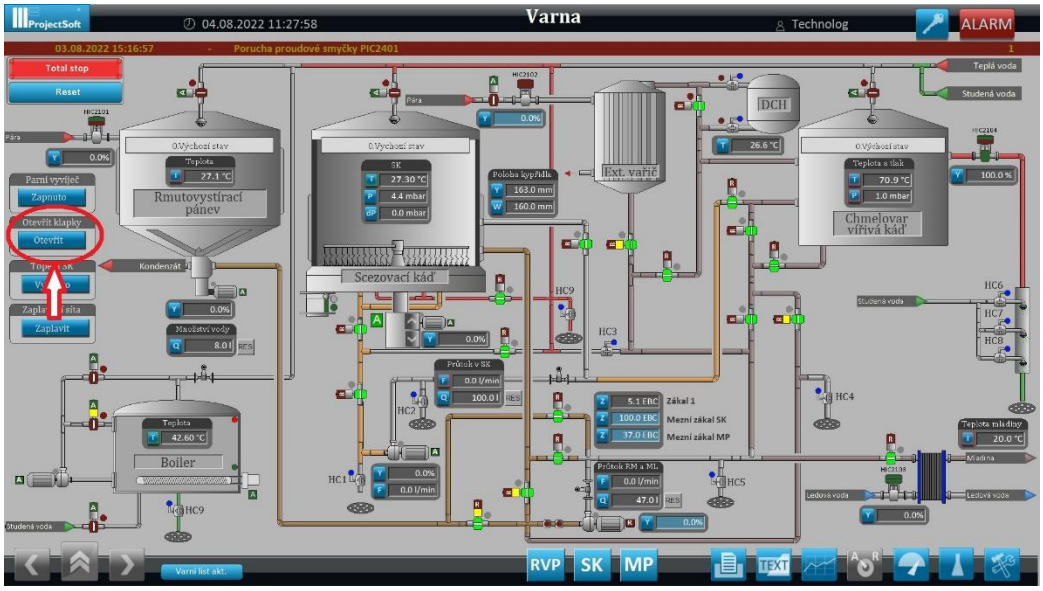


Figure 26 - Release of the wort from the system through the "Project Soft" programme.

Lastly, all the equipment in the system was cleaned manually with water and the help of chemical products. All the valves remained open in order to have a sanitized system for the future experiments. The chemical products used were: P3-HOROLITH, P3-STERIL and P3-OXONIA.

3.5. Sample Analysis

The sample analysis has been made in the laboratories of the Institute. After taking the samples out of the freezer, they were put in a warm bath water so that the ice formed in it melted.

Each of the samples were filtrated into a volumetric flask, using a glass funnel and filter paper, as it is represented in Figure 27. After the filtration, the filtered liquid was placed once again in the tubes for its analysis.

Figure 27 - Filtration of the samples though a filter paper.

With all the samples in liquid form, it was measured its pH with a pH meter, and density with an alcohol analysing system "Anton Paar – Alcolyzer Beer ME" in order to perceive if all of them are according to what was expected, without biological transformations in it. The pH of the samples from 0 to 90 minutes must stay around the same value (6 - 7), and the density of all of them depended on the degree to which the beer was being brewed.

The samples regarding the investigation of gluten content were taken to analyses out of the institute and the results were obtained by absorbance.

The samples regarding the analyses of α-acids content were done following the protocol "Analytica-EBC - 7.8 - Iso-α-, α- and β-Acids in Hop and Isomerised Hop Extracts by HPLC". The procedure was executed using a vacuum pump and extraction columns in order to obtain the extract in 5 mL volumetric flasks, as represented in Figure 28. The obtained samples were transferred to a clear sampling vial for the analysis by HPLC-UV.

Figure 28 - Extraction of the components through extraction columns.

3.6. HPLC analyses

The chromatography via HPLC-UV took place in the HPLC apparatus, and it was performed also according to the protocol "Analytica-EBC - 7.8 - Iso-α-, α- and β-Acids in Hop and Isomerised Hop Extracts by HPLC" (anonymous protocol). The apparatus quantified α-acids and iso α-acids that were presented in each vial sample. All the samples must be identified in order to know which results are from which sample.

In the HPLC apparatus, compounds from a sample are separated taking into account their distinct physical and chemical interactions with the two phases of the chromatographic system - mobile and stationary phase. The mobile or eluting phase travels through the system and carries the components of the mixture. The stationary or fixed phase is held by a support and holds the sample substances as they pass through the system. (Hage, 2018).

Compounds that have strong interactions with this phase are retained and move more slowly through the system. Conversely, compounds with weak interactions with the stationary phase spend more time in the mobile phase. Different retention times in the stationary phase generate different peak areas, which are associated with a certain compound. Those peak areas were later converted into concentrations.

HPLC chromatography is based on refractive index, with a refractive index detector, as the mobile phase passes through a cell, changes in the refraction of light from substances are evaluated. Absorption detectors measure the absorbance of light at a wavelength. In fluorescence detectors, the components pass through a cell that signals the existence of fluorescent compounds (Hage, 2018).

With these results it was possible to determine the percentage of α-acids that were isomerized (Appendix B).

4. Results and Discussion

For all the experiments performed the amount of alpha acids present in hops was 93.22 mg/L. In all of them the concentrations of all alpha-acid homologues and their isomers were determined, in order to determine the isomerization yield.

The values obtained for each sample (corresponding to a certain time of the experiments) are the result of the analysis of two duplicates in some experiments. However, the values obtained for the two replicas are extremely similar, and the associated errors are not represented.

4.1. Wort boiling without cavitation: Traditional process

Isomerization of alpha-acids into iso-alpha acids tend to occur in temperatures around 100 °C during wort boiling, in traditional processes. The Table 2 below, represents an experiment where it was tested the isomerization without the use of HC during 90 min at a temperature of 100 °C, using hop extract.

Table 2 – Data relative to the wort boiling experiment at 100 °C without HC

Sample (min)	*co-humulone*	*n+ad-humulone*	*Σ Alfa-acids (mg/L)*	*iso co-humulone*	*iso n-humulone*	*iso ad-humulone*	*Σ Iso-Alpha acids (mg/L)*
0	13.1	17.41	30.51	1.87	2.13	0,54	4,53
15	11.53	15.18	26.71	4.94	5.07	1.22	11.23
30	9.28	12.42	21.7	6.91	6.69	1.49	15.08
45	8.48	11.23	19.71	10.35	9.77	2.23	22.35
60	6.63	8.82	15.45	10.4	9.89	2.24	22.53
75	5.44	7.33	12.77	12.67	11.98	2.65	27.3
90	4.69	6.37	11.07	12.59	11.92	2.61	27.11

During the 90 min, the concentration of alpha acids tends to decrease contrasting with the concentration of iso-alpha acids which increased along the experiment. This occurred as was expected since the alphas, at such a high temperature, will convert into iso-alphas, turning the degradation of one into the formation of the other.

Even though, the quantity of hop extract alpha-acids placed into the medium was 93.22 mg/L, looking at the Table 2, the value noted at the beginning of the experience was 30.51 mg/L. This can be explained

because there could be losses of alpha acids by adhesion to vessel walls and to the wort system due to its low solubility in water, according to Malowicki and Shellhammer (2005). This initial value of alphas is basically the same (30-40 mg/L) for all the experiments executed, inclusive with hydrodynamic cavitation.

This way, only about 30 % of the alpha acids placed in the medium (93.22 mg/L) were registered in the final form of their isomers (27.11 mg/L), which is according to what is expected, since according to Jaskula et al., (2008) only about a third of the alphas put in the medium appear in the iso-alphas form at the final of wort boiling (utilization).

From the formula in Equation 5, IBU represents the quantity of iso-alpha acids per litre of the solution in the medium, and for this experience that value is 30 mg/L. As its possible to observe, it was obtained a value of 27.11 mg/L in the end of the experiment which was very close to what was expected. Although, this value should be higher because the 30 mg/L of iso-alpha acids is expected to be achieved in the final beer, and that way, the concentration obtained (27.11 mg/L) would be a bit lower if the fermentation took place. This probably means that the efficiency of the brewery is lower than it was expected.

The total isomerization was calculated using the formula described in the Appendix A.3, and in this case, it was registered a total isomerization of 53.7 %. This value is in agreement to what is expected, because many studies (Caballero et al., 2012) already referred before (Caballero et al., 2012), appoint no more than 60 % of isomerization during a normal wort boiling.

The next Figure 29 shows the isomerization during the 90 min of the experiment.

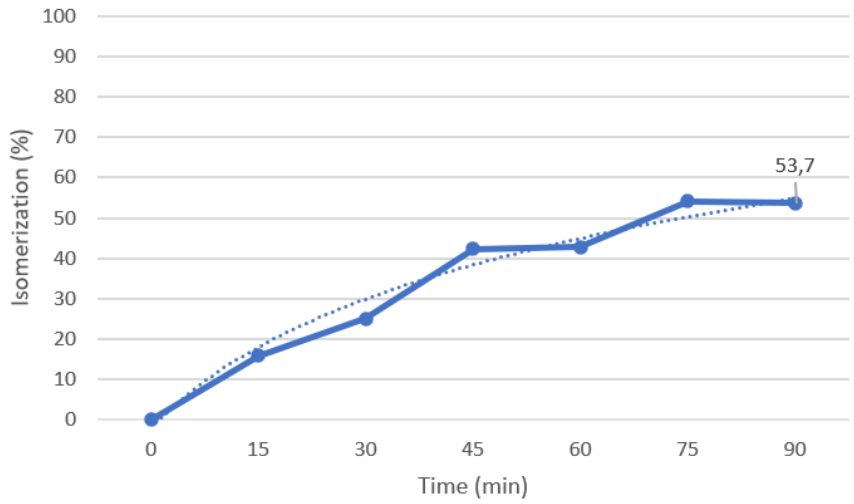


Figure 29 - Isomerization during 90 min for the experiment at 100 °C without HC.

From the Figure 29 it is possible to observe that most of the isomerization occurs at the beginning of the experience, in the first 45 min, leaning to have a logarithmic tendency which is corresponding to what is expected for a traditional process according to Hertel & Dillenburger (2010).

This probably happens because in the initial part of the experiment it does not exist a medium equilibrium, since at the beginning, there is a huge number of alpha acids and there are no or little iso-alphas. During time, these concentrations will get to a certain equilibrium and the isomerization yield should be lower. At the end the isomerization seems to be stabilizing, indicating that after the 90 min, no more alphas would isomerize.

4.2. Pellets vs Extract

In order to understand if the best raw material to optimize isomerization in hydro cavitation experiments would be hop pellets or hop extract, two experiments were conducted with the same temperature at 70 °C and 0.15 cavitation number to see which material is capable to obtain higher isomerization yields.

Figure 30 shows the isomerization occurred during 90 min of experience for both hop applications.

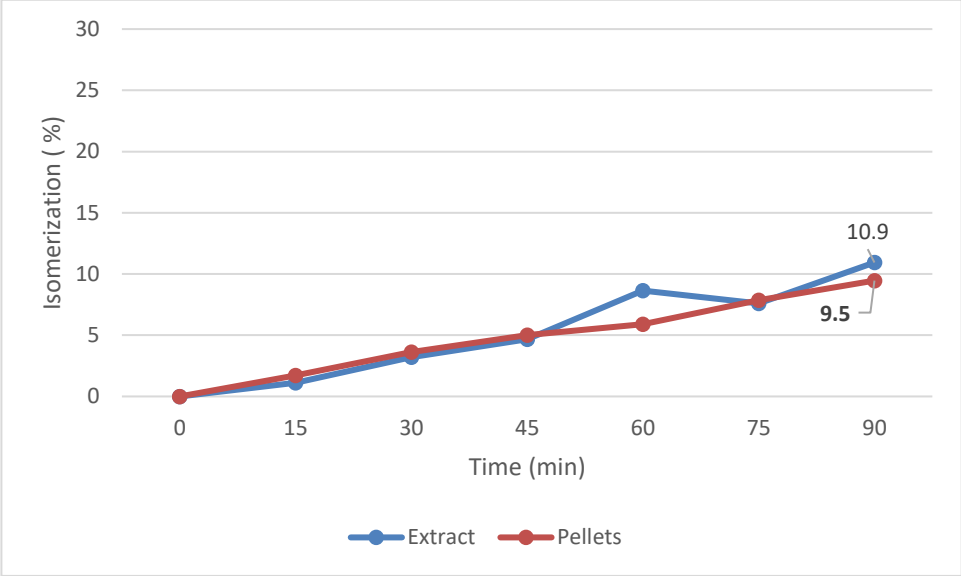


Figure 30 – Isomerization for the experiments using hop extract and hop pellets (70°C, CN=0.15).

Even tough, the conditions on which the experiments were performed are not the best ones to promote a better isomerization, because it was executed at a low temperature, hop extracts had the capability to achieve higher isomerization. Hop extract achieved 10.9 % isomerization and hop pellets achieved a lower isomerization of 9.5 %. The difference between the two results is not very significant but indicates that using one or another the isomerization rates will be very close. The results support the expectation that, hop extract promotes more utilization of alphas and dissolve faster in water. Besides that, hop pellets are in the solid form which can retain some valuable content in it (HAAS, 2016).

All the experiences which will follow on the next topics were performed using hop extract.

4.3. Isomerization without wort boiling

As it was referred before, isomerization happens usually when temperatures are around 100 °C, during wort boiling. In order to see, if it's possible to obtain some isomerization at temperatures lower than that value with HC, two experiments were executed at 70 °C with and without the use of the cavitation technology.

The Table 3 below, shows the data obtained for the experiment at 70 °C without cavitation.

Table 3 - Data relative to the experiment performed at 70 °C without HC

Sample (min)	*co-humulone*	*n+ad-humulone*	*Σ Alpha acids (mg/L)*	*iso co-humulone*	*iso n-humulone*	*iso ad-humulone*	*Σ Iso-Alpha acids (mg/L)*
0	10.29	18.51	28.81	1.54	0.00	0.00	1.54
15	10.59	19.33	29.90	2.15	0.03	0.28	2.46
30	11.36	20.15	31.49	1.53	0.03	0.30	1.86
45	10.64	18.75	29.37	1.48	0.03	0.34	1.85
60	12.21	20.94	33.14	1.98	0.07	0.46	2.50
75	11.23	19.13	30.34	2.62	0.09	0.51	3.23
90	10.51	17.66	28.16	1.12	0.10	0.49	1.71

As it is possible to see from Table 3, it is not perceptible an increase of iso-alpha acids during the 90 min of the experiment. It was detected a small concentration of it, which probably were capable to isomerise at such low temperatures or could also be some residual iso-alphas present in the hop extract. Besides that, the concentration of alpha-acids during time stayed basically the same.

The next Table 4 represents the concentration of alpha and iso-alpha acids during 90 min of the experiment for 70 °C and 0.1 cavitation number.

Table 4 - Data relative to the experiment performed at 70 °C with 0.1 CN

Sample	*co-humulone*	*n+ad-humulone*	*Σ Alfa-acids (mg/L)*	*iso co-humulone*	*iso n-humulone*	*iso ad-humulone*	*Σ Alfa iso-acids (mg/L)*
0	10.67	16.10	26.78	0.24	0.35	0.13	0.71
15	21.21	29.75	40.00	0.77	1.01	0.33	2.11
30	14.71	21.73	36.44	0.87	1.32	0.42	2.61
45	17.65	25.42	43.07	1.10	1.66	0.51	3.28
60	15.37	22.86	38.23	1.58	2.46	0.74	4.78
75	14.32	20.81	35.13	2.15	3.42	1.01	6.57
90	13.91	19.96	33.87	2.68	4.32	1.26	8.25

In this experiment, the isomerization of alpha acids was tested during 90 min and observing the table it is notable that a small concentration of alpha acids was isomerized. The total isomerization obtained was 16 %. Even though, this value is considerably low, it is a very positive result since the experiment was carried out at 70 °C, a temperature at which there is no isomerization in traditional methods without cavitation.

Figure 31 shows the formation of iso-alpha acids during the time the two experiments were tested.

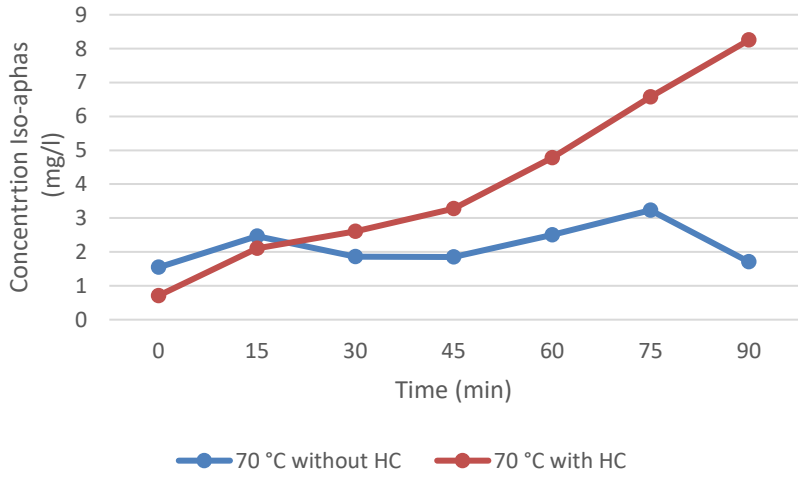


Figure 31 - Iso-alphas formation for the experiments at 70 °C without HC and with 0.1 CN.

On the previous graphic from Figure 31, it is visible the difference between the two experiments, with a clear increase of the concentration of iso-alpha acids in the experiment with cavitation. The data

collected for the experiment without cavitation, even though it is enough to observe that formation of iso-alphas does not seem to take place with time, it seems that the values are unstable, with increases and decreases of values which is not supposed to happen. This may have happened basically because isomerization does not take place, and so, with small analytical errors of measurements in some samples, this value could increase or decrease a bit (2 mg/L) and make a big difference in the final results.

These differences between the two experiments are fundamental to understand something very important: it is possible to obtain isomerization below temperatures of wort boiling (100 °C) with the energy originating from hydrodynamic cavitation.

4.4. Variation of alphas and Iso-alphas during time

Isomerization, in a few words, consists of a transformation of alpha-acids into iso-alpha acids. Therefore, and as it was concluded before, the concentration of alphas tends to decrease during time contrasting with the increase of iso-alphas. In order to see how HC affects these concentrations during time, inclusive to all the types of alpha acids (co-humulone, n-humulone and ad-humulone) and its isomers, the data referred before about the experiment at 70 °C with cavitation number 0.1 is aborded in detail in the next topics, as well as at 90 °C with same cavitation number in order to have a term of comparison at different temperatures.

4.4.1. General overview

As it is possible to see from the Figure 32 related to the experiment at 90 °C, and as it was expected, also with the use of the HC the concentration of alpha acids tends to decrease with time, and their isomers tend to increase.

Figure 32 shows that relationship tendency more clearly and the sum of both during time.

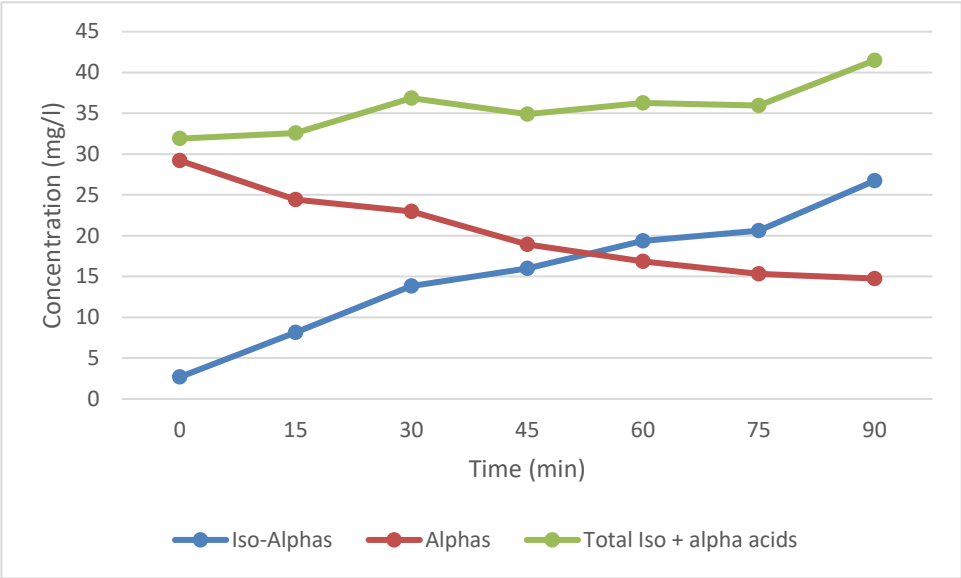


Figure 32 - Variation of alphas and iso-alphas during 90 min for the experience (90 °C, CN=0.1).

Observing the Figure 32, it is possible to see a small concentration of iso-alpha acids in the first sample (0 min). This concentration does not enter to the isomerization calculations, since the sample is taken right at the moment the hydrodynamic cavitation starts. This concentration could be due to the effect of the temperature, or some residual iso-alpha acids present in the extract and is present in all experiments executed.

The sum of the concentrations of alpha and iso-alphas seems to increase during time. This information does not seem normal at a first sight, since iso-alphas are isomers of alpha acids (same molecular weight), which would make the final concentration very close to the initial concentration, since what is isomerized in alphas is transformed into iso-alpha acids. Also, according to some studies (Jaskula et al., 2008), the isomerization seems to be a first order kinetics reaction. Besides that, a decrease and not an increase of the values would be more expected since there might be some losses to the medium.

One hypothesis for that increase, is the low solubility of alpha acids. According to Kunze (2004a), the alpha acids have a very low solubility until achieving high temperatures and isomerize into the iso-alpha acids, which are much more soluble. Moreover, Malowicki and Shellhammer (2006) have done a study in which concludes that, for different pH in the medium, during wort boiling, the concentration of iso-alpha acids in all of them were basically the same during time. In the other other hand, the concentration of alpha acids showed considerable differences during the time of different experiences. This happened because the solubility of alpha acids is very low. This means that, in wort, the alpha acids would have been completely in solution and contributing for isomerization, but only the most soluble ones are

detected by HPLC. In the current investigation, the quantification of alpha acids also occurred via HPLC, and so, not all of them are being counted.

In other words, in the wort exists essentially, more and less soluble alpha acids in which the first ones are transforming into their isomers. When the experiment initiates, the conversion of alpha acids into iso-alpha acids starts with the more soluble alphas isomerizing. While this is happening, the less soluble alphas, that are on the walls of the reactor, are still dissolving into the water. As the experiment progresses, some of these low soluble acids end up to dissolve, entering to the total number of alpha acids present in the medium. Thus, the sum of iso-alphas and alphas over time tends to increase.

These variations tend to happen in all the experiments and could be seen in the tables and graphics from the Appendix C. However, the solubilization of the alpha acids end up having a different impact in different temperatures because the higher it is the temperature, the higher is the solubilization. With that in mind, for the experiments at low temperatures, mainly at 70 °C, the concentrations discussed in Figure 32 (previous one), are in many cases very irregular.

Figure 33 represents the concentrations for the 90 min at 70 °C.

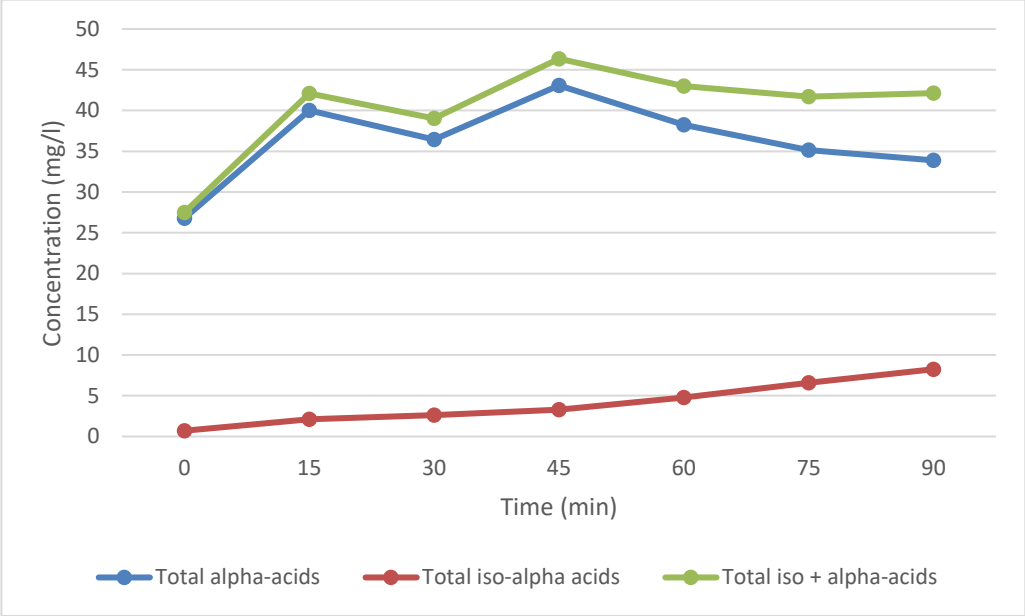


Figure 33 - Variation of alphas and iso-alphas during 90 min for the experience (70 °C, CN=0.1).

First of all, it is clear to see that the concentration of iso-alphas increases almost linearly until the end, however the concentrations of alphas seem to have certain irregularities. Usually, the iso and alpha concentrations tend to increase or decrease uniformly. Still, at lower temperatures, this does not always

seem to happen, especially for alphas as it is seen in the Figure 33, which again, is related with the low solubility particularly at lower temperatures.

Besides the solubility, the irregularities detected (highs and lows) for both alpha and the summatory concentrations are related to the isomerization. In this case, for example, and for low temperatures in general, the formation of iso-alphas is lower. This small conversion of alphas into iso-alphas, during a period of time in which a considerable higher quantity of soluble alphas is able to isomerize, is enough to provoke these anomalies. Besides that, those anomalies could be associated with analytical errors, for example: in the Appendix C.9, the sample at 75 min there is a decrease of iso-alphas (not supposed to happen) and an excessive decrease of alphas. Both values seem to return to the expected in the final sample. Another example is the Appendix C.11, where the initial concentration of alphas detected seem to be too high when compared with all the other experiments, which is reinforced by the next samples which have almost half of the concentration of the first one, while the concentration of iso-alphas stayed around the same number. In some other experiments of the study these anomalies happened and can see observed in the Table and Figures of Appendices C2, C4 and C5 (experiments at 70°C)

In this current experiment, from 45 min onwards the sum of iso and alpha acids stays more or less the same. This occurs since the formation of iso-alphas is very low and from this point probably there are no more insoluble acids solubilizing in the water, therefore, what is formed from alphas is converted into their isomers.

4.4.2. Alpha acids

Figure 34 below, shows the variation of the concentration of alpha acids during time with the application of HC for 90 °C with 0.1 cavitation number. All the other experiments have a similar trend and can be seen in the graphics of Appendix C.

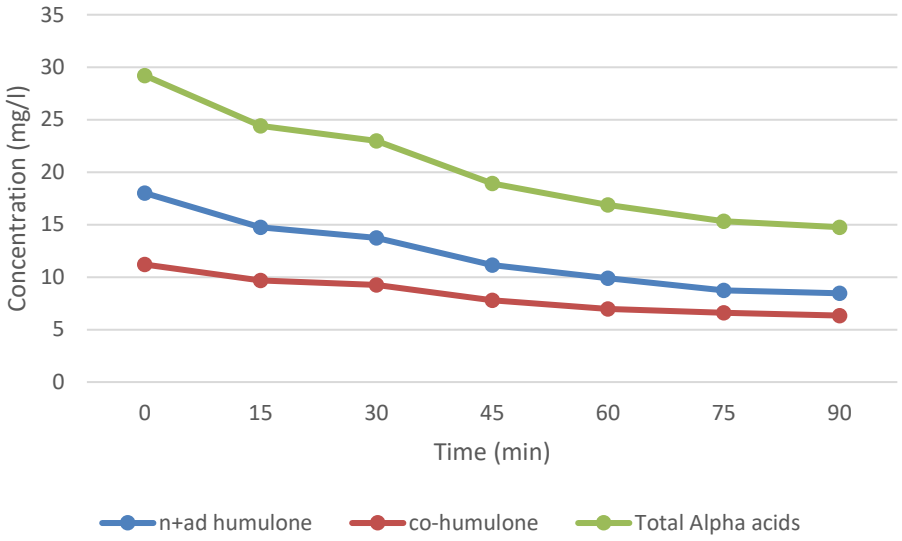


Figure 34 - Variation of the alpha acids homologues during the experience (T=90°C, CN=0.1).

It depends on the variety of the hop, but normally, the portions of alpha acids stand by 10 - 15 % ad-humulone, 20 - 65 % co-humulone, and 35 - 70 % n-humulone (Kostrzewa et al., 2016). Observing the figure above, it is possible to conclude that each concentration of alpha acid is according to what was expected. The HPLC counted n-humulones and ad-humulones together, and so it was not possible to determine the precise concentration of each alpha acids. Nevertheless, the concentration of co-humulones corresponds to around a third of the total alpha acid concentration which is a percentage in agreement with the literature.

The percentage of n-humulones is expected to be more or less the same (maybe a bit higher) as the percentage for co-humulones. That seems to happen since n-humulones and ad-humulones together make a concentration bit higher than the co-humulones alone by themselves.

These conclusions are equal to all the experiments (with different conditions) tested, including with traditional brewing, which means, hydrodynamic cavitation does not interfere with different alpha acid homologs consumption.

4.4.3. Iso-alpha acids

Figure 35 shows the variation of the concentration of iso-alpha acids during time with the application of HC. As it was expected its concentration increases during time.

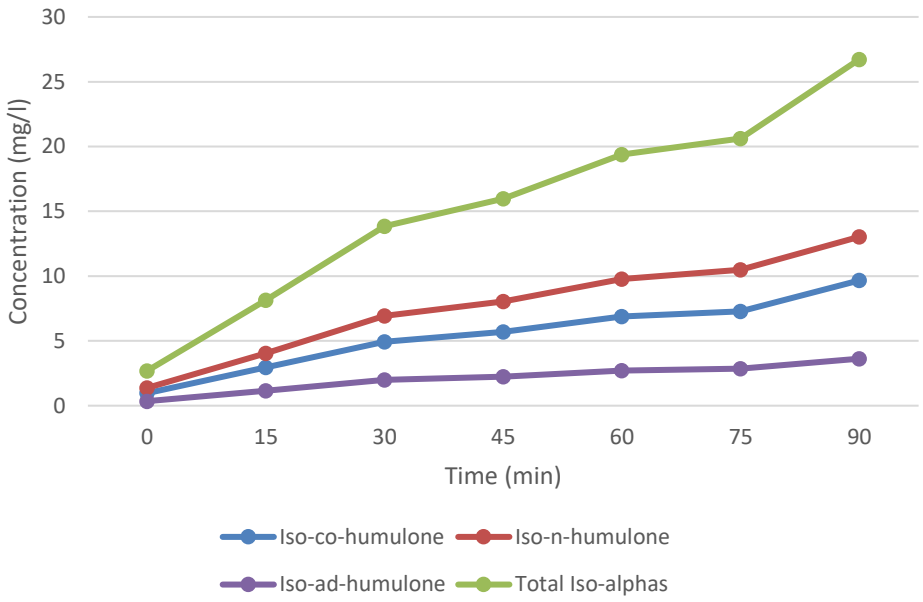


Figure 35 - Variation of the iso-alpha acids homologues during the experience (T=90°C, CN=0.1).

Iso-alpha acids are isomers of alpha acids, so the results of the concentrations should be in line with the concentrations of alpha acids. For example, co-humulones corresponded to around a third of the total alpha concentration, thus, its isomer iso-co-humulone, also correspond to about a third of the total iso-alpha concentration (Figure 35).

Besides that, this Figure also helps to understand which alpha acid had higher concentration in the medium between co-humulone and n-humulone. Since the concentration of iso-co-humulone is a bit higher than iso-co-humulone, it is possible to conclude that, probably in the medium, there was a higher concentration of n-humulones when compared with the co-humulones. The isomer iso-ad-humulone presented the lowest concentration among iso-alphas such as ad-humulone among the alpha acids.

All the other experiments have a similar trend and could be seen in the Appendix C.

4.5. Influence of temperature

As it is already known, in traditional beer production, isomerization of bitter alpha acids occurs during wort boiling where high temperatures are used in order to speed up this phenomenon. It is necessary to apply these high temperatures in order to achieve the activation energy for the transformation of alpha acids into iso-alpha acids to happen (Malowicki & Shellhammer, 2005).

These following experiments are displayed in this topic in order to prove that as higher it is the temperature applied for certain cavitation number, higher will be the percentage of isomerization.

Figure 36, represents three experiments executed at three different temperatures (70, 80 and 90 °C), applying the same cavitation number of 0.1 in the process.

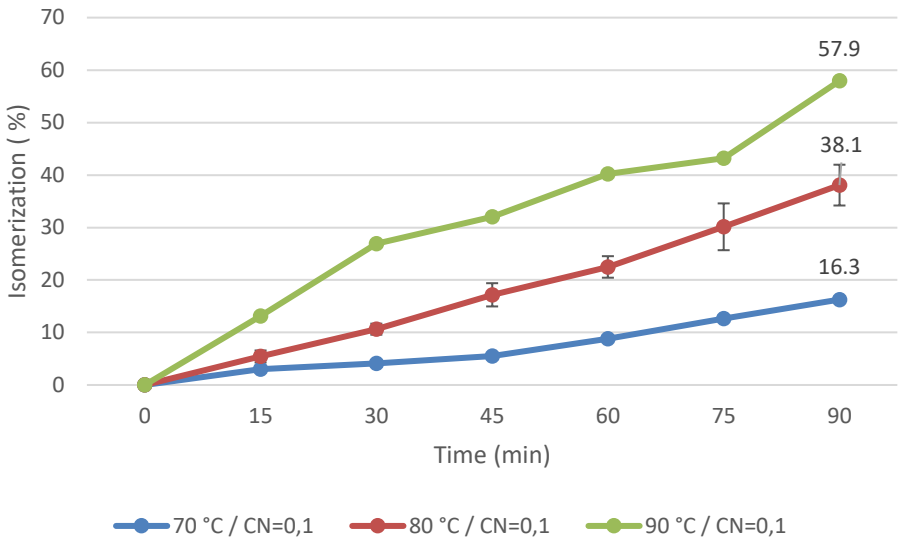


Figure 36 - Isomerization for the experiments at 70, 80 and 90 °C with CN=0.1.

Isomerization was clearly higher for the superior temperature tested, in this case for 90 °C, with a total percentage of 57.9 % alpha acids isomerized. In a traditional brewery, to achieve the activation energy of isomerization, temperatures of boiling water 100 °C are used. Since at 70 °C with hydrodynamic cavitation it is already possible to achieve some isomerization (Topic 4.3), it is indeed expectable that at 90 °C that value will be even higher.

The experiment at 80 °C was executed twice in order to see the variation error of the results. Even with the associated error, the tends follow the same path without interfering with the other results. The final isomerization achieved at 80 °C was 38.1 % ± 3.9.

4.6. Influence of the cavitation number

It is already known that the isomerization increases at higher temperatures. In order to see, the impact of the cavitation number at a certain temperature, the data from all the temperatures tested at different cavitation numbers is organized in the next Figure 37.

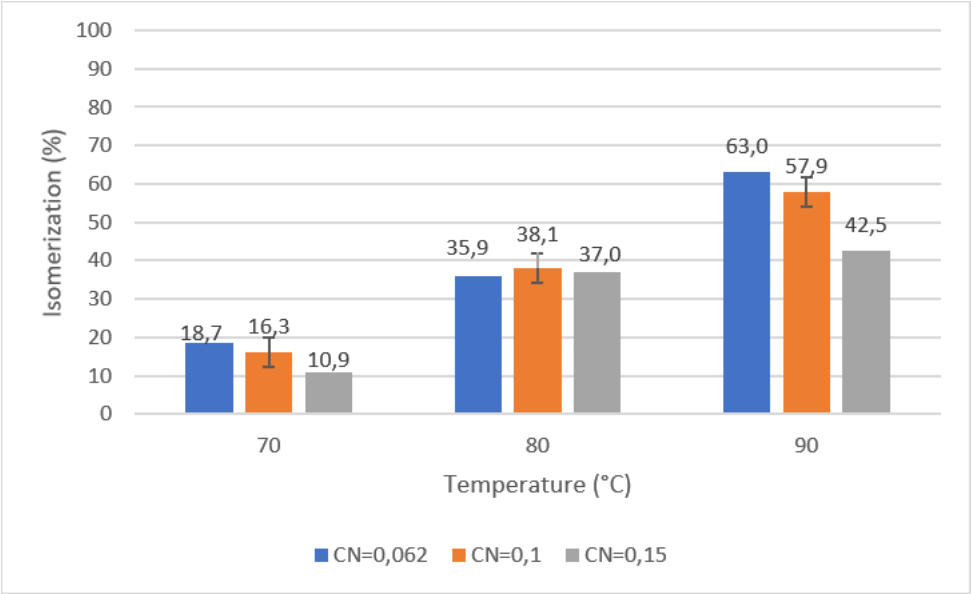


Figure 37 - Influence of the CN on isomerization for different temperatures.

First of all, and considering the last topic, it is visible that independent of the cavitation number applied, the higher the temperature, the higher the isomerization is.

Looking at the Figure 37, at 90 °C there is very clear that isomerization increases with decreasing cavitation number. At lower cavitation number there are more cavities being formed, and more collapses. At higher cavitation number, there are less cavities being formed, which results in fewer alpha acids being captured by them and isomerized.

The highest isomerization (63 %) was obtained at 90 °C applying 0.062 cavitation number. This was higher than the isomerization during traditional boiling (53.7 %)

The lower temperatures used, 70 and 80 °C, turned down to limit the effect of the cavitation, and at these temperatures is very difficult to find a trend in the results. Despite the results for 70 °C seem to have a trend similar to the experiments at 90 °C, the calculations for this temperature made use of values at different times of the experiment (the highest value of the sum alphas with iso-alphas was not the final one) due to the low solubilization of the medium.

All the isomerizations achieved for the different cavitation numbers at those temperatures are very alike, with small differences between them. Therefore, at lower temperatures a trend in the results is not detected and it is only possible to conclude that there is a possibility of achieving isomerization at temperatures significantly lower than boiling using hydrodynamic cavitation.

In order to find a possible trend in the results for 70 and 80 °C, more experiments should have been done. The only experience that was done twice was for 80 °C with 0.1 cavitation number, and the

associated error is represented in Figure 35, and this proves that for this temperature, the studied range of cavitation numbers has no significant effect on the isomerization.

4.7. The ideal situation

All the experiments with or without HC are presented in Table 5. The experiments were tested for different temperatures with different cavitation numbers in order to see, what were the conditions that can optimize the isomerization of alpha acids.

Table 5 - Presentation of all the results of the study

		Cavitation number			
		———	0,062	0,1	0,15
Temperature (°C)	100	53,7%			
	90		**63,0%**	57,9%	42,5%
	80		35,9%	38,1% ± 3,9	37,0%
	70	0,0%	18,7%	16,3%	10,9% / 9,5%

Without HC
Extract w/ HC
Pellets w/ HC

The range of temperatures tested for the experiments was chosen considering the typical temperature used in traditional boiling for isomerization (100 °C) and temperatures below that (70-90 °C) in order to see the possibilities of isomerization under those conditions. The range of the cavitation number goes from the lowest number the pump could operate (0.062) until the value at which the isomerization seems to start to decrease (0.15).

First of all, the results show that with the use of hydro cavitation, regardless of temperature, cavitation number or hop type, it is possible to achieve isomerisation levels of alpha acids.

From the experiments executed, the one which presented the highest percentage of isomerization was at 90 °C and a cavitation number of 0.062 with a total isomerization rate of 63 %. This number was

higher compared with the one obtained for 100 °C (53.7 %) without hydrodynamic cavitation. Besides that, the isomerization obtained at the same temperature (90 °C) for the higher cavitation numbers were also promising, indicating that the use of the technology at this temperature is recommendable. In other words, compared to traditional wort boiling at 100 °C for 90 minutes (case tested in this study), it is possible to obtain higher isomerisation rates at 90 °C (which is not a boiling temperature) by applying hydrodynamic cavitation, which is a difference of 10 °C less being applied. This could be very interesting, as there is a possibility of energy savings during wort boiling in beer production. According to Slawitsch et al. (2011), the energy demand in traditional wort boiling is very high (24-54 MJ/hl), this way, if the temperatures applied during this stage are lower, the energy consumption will also be lower.

In brewing, one important portion of the costs are related to energy costs, and wort boiling is one of the stages which consumes the highest amount of energy. Using hydrodynamic cavitation, no boiling temperatures are needed, and that way, it is possible to maintain the wort volume without losses though evaporation while saving energy during the process. This is very important, since according to Kubule et al. (2016), 93 % of heat losses happen during traditional wort boiling.

4.8. Gluten

To see the potential influence of HC in the reduction of gluten content in beer, a single test was carried at 70 °C and a cavitation number of 0.1 and the results were obtained by absorbance. Even though, the most important step in brewing to prevent gluten content is fermentation, this test was carried out during wort boiling, in order to see if it is possible to reduce the concentration of gluten relative proteins before reaching fermentation.

The final gluten content will depend on the assimilation of the amino-acid proline, which is a component of gliadin, a group of proteins responsible to the final gluten content beer (Albanese et al., 2017b)

Figure 38 shows the concentration of gliadin across the 90 min of the experiment.

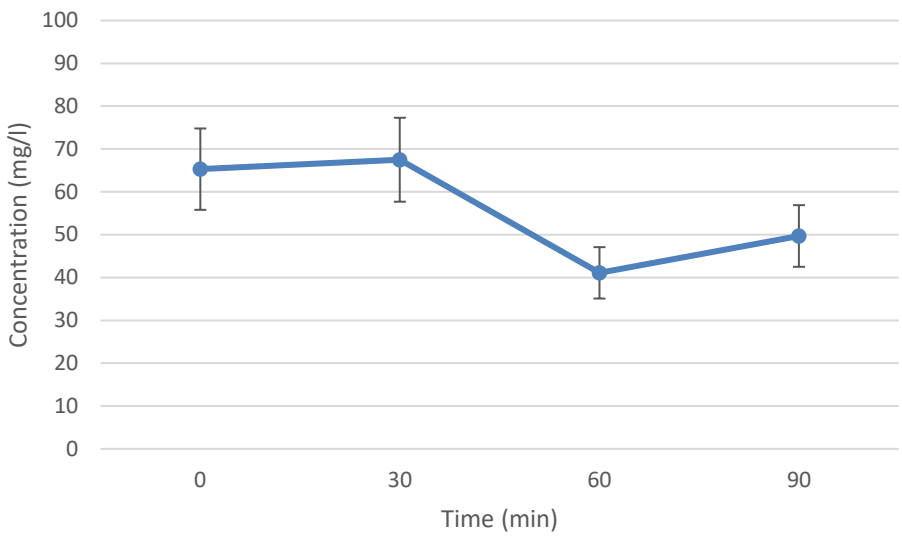


Figure 38 - Gliadin concentration during the experience.

From the previous graphic, the concentration appears to decrease during the time of the experiment, however, it would be expected this decrease to be progressive, without any increase in concentration as shown in the figure. This is probably due to the high tolerance intervals in the concentration values that are also represented in the figure. So, it is expected that the concentration decreases constantly over time.

The initial value of concentration was 65.3 ± 9.5 mg/L and the final one was 49.7 ± 7.2, which proves that is possible to reduce the gluten content during wort boiling with hydrodynamic cavitation, applying a low temperature of 70 °C.

5. CONCLUSIONS AND RECOMMENDATIONS

The main objective of this study was to understand the effect that hydrodynamic cavitation has on the wort boiling stage. To be precise, to understand the effect of this innovative technology on some constituents of the wort, with a special focus on the isomerization of alpha acids when compared with traditional methods. Preliminary measurements focused on the effect of cavitation on wort gluten content were also carried out.

Regarding the isomerisation of alpha acids, the tests carried out showed that the use of hop pellets or hop extract does not make much difference in the sense of obtaining a higher isomerisation percentage. However, the results support the expectation that, hop extract promotes more the utilization of alpha acids. Furthermore, it was proven that it is possible to obtain isomerization below temperatures of wort boiling (100 °C) with the energy originating from hydrodynamic cavitation. At 70 °C with the use of the HC technology some isomerization was observed, contrasting with the results at the same temperature without HC. Besides that, cavitation seems not to have a different impact on alpha acids homologues and their isomers, when compared with a traditional method.

It was also proven that using hydrodynamic cavitation, and independently of the cavitation number, the isomerization tends to increase with the applied temperature. The highest isomerization (63 %) was obtained at 90 °C applying 0.062 cavitation number. This was higher than the isomerization obtained during traditional wort boiling (53.7 %). The results have shown that the use of 90 °C with HC, regardless the cavitation number, seem very promising to achieve better results than traditional boiling. No boiling temperatures are needed, and that way, it is possible to maintain the wort volume without losses though evaporation while saving energy during the process.

The isomerization achieved for 70 and 80 °C were very alike, with small differences between them due to the low solubility of alpha acids in the medium. Therefore, at lower temperatures a trend in the results was not detected and it is only possible to conclude that there is a possibility of achieving isomerization at temperatures significantly lower than boiling using hydrodynamic cavitation. In order to find a clear trend at these temperatures, it is recommendable to do more experiments to find associate errors.

In the future, it will be necessary to evaluate, in addition to the isomerization of alpha acids, other qualitative parameters of the wort obtained by combined heating and hydrodynamic cavitation and to compare them with wort from traditional wort boiling. This research will be focused on the parameters expressing thermal damage of the wort and also on sensory active substances.

For gluten, the results shown that hydrodynamic cavitation has an impact, and it was possible to verify a decrease in the concentration of gliadin in the medium. At the beginning of the experiment was detected a concentration of 65.3 ± 9.5 mg/L and at the end was detected 49.7 ± 7.2 mg/L.

To conclude, hydrodynamic cavitation seems to be a very promising technology in the brewing sector, making it possible to improve the isomerization of bitter alphas acids (contributing to the final taste), as well as the reduction of gluten in the final beer (health concern). All of this by using lower temperatures in the process, contributing in a very positive way to energy savings in the process.

REFERENCES

Albanese, L. & Meneguzzo, F. (2019). Hydrodynamic cavitation – assisted processing of vegetable beverages: Review and the case of beer-brewing. In Grumezescu, M. & Holban, A. M. (Eds). *Production and Manegement of Beverages.* (pp. 211-257). Woodhead Publishing.

Albanese, L., Ciriminna, R., Meneguzzo, F. & Pagliaro, M. (2017a). Beer-brewing powered by controlled hydrodynamic cavitation: Theory and real-scale experiments. *Journal of Cleaner Production*. 142 (2017), 1457-1470.

Albanese, L., Ciriminna, R., Meneguzzo, F. & Pagliaro, M. (2017b). Gluten reduction in beer by hydrodynamic cavitation assisted brewing of barley malts. *Food Science and Technology*. 82(2017), 342-353.

Anonymous & Berry, A. (2022, January 11th). *Cones, pellets, oils... What is the best choice for my beer.* Hop Store. https://www.hopstore.fr/en/formes-de-houblons/

Bamforth, C. (2003). Grain to glass: the basics of malting and brewing. In *Beer: Tap Into the Art and Science of Brewing.* (2nd ed., pp. 49-64). Oxford University Press.

Brányaik, T. & Pires, E. (2015a). An overview of the brewing process*. In Biochemistry of Beer Fermentation.* (pp. 1-8). Springer.

Briggs, D. E., Boulton, C. A., Brookes, P. A. & Stevens, R. (2004a). Malts, adjunts and supplementary enzymes. In Brewing: *Science and practise*. Woodhead publishing.

Briggs, D. E., Boulton, C. A., Brookes, P. A. & Stevens, R. (2004b). Water, effluents and wastes. In Brewing: *Science and practise*. Woodhead publishing.

Caballero, I., Blanco, C. A., Porras, M. (2012). Iso- α -acids, bitterness and loss of beer quality during storage. *Trends in Food Science & Technology*. 26, 21-30.

Carpenter, J., Badve, M., Rajoriya, S., George, S., Saharan, V. K. & Pandit, A. B. (2016). Hydrodynamic cavitation: an emerging technology for the intensification of various chemical and physical processes in a chemical process industry. *Reviews in chemical engineering*.

Cela, N., Condelli, N., Marisa, C.C., Perretti, G., Cairano, M.D.,Tolve, R., Galgano, F. (2020). Gluten-Free Brewing: Issues and Perspectives. *Fermentation*. 6,53. 1-28.

Craft Beer & Brewing. (2020). Malt your own barley. https://beerandbrewing.com/malt-your-own-barley/

Dunn, A. R. (2006). Packaging technology. In Priest, F. G. & Stewart, G. G. (Eds). *Handbook of Brewing.* 2nd ed. pp 565-604). Taylor & Francis group.

Eßlinger, H. M. (2009). Fermentation, maturation and storage. In Eßlinger, G. M. (Ed). *Handbook of brewing: Processes, technology, markets.* (pp 207-224). Wiley-VCH.

Eumann, M., (2006). Water in brewing. In Bamforth, C. W. (Ed). *Brewing new technologies*. (pp 183-207). Woodhead publishing limited.

Franc, J. P. (2007). The Rayleigh-Plesset equation: a simple and powerful tool to understand various aspects of cavitation. In Agostino, L. & Salvetti, M. V. (Eds). *Fluid dynamics of cavitation and cavitating turbopumps.* (pp. 1-43). SpringerWienNewYork.

Franklin Electric. (2022). MH Series Horizontal Multistage. https://franklinwater.eu/products/surface-pumps/fps/fps-multistage-pumps/mh-series-horizontal-multistage/

Gogate, P. R., Tayal, R. K. & Pandit, A. B. (2006). Cavitation: A technology on the horizon. *Current science*. 91, 35-46.

Goiris, B. J., Aerts, G., Cooman, L. C. (2010). Hop α -acids isomerisation and utilisation: an experimental review. *Cerevisia*. 35, 57-70.

HAAS. (2016, September 26). Best practises guide pure resin CO_2 hop extract. https://www.johnihaas.com/news-views/best-practices-guide-pure-resin-co2-hop-extract/

Hager, A. S., Taylor, J. P., Waters, D. M. & Arendt, E. K. (2014). Gluten free beer - A review. *Food Science & Technology.* 36, 44-54.

Hertel, M. & Dillenburger, M. (2010). Measures for raising yield beer brewing (Part 1*). BRAUWELT INTERNATIONAL*. 92-95.

Huang, Y., Tippmann, J., Becker, T. (2013). Kinetic Modeling of Hop Acids during Wort Boiling. *International Journal of Bioscience, Biochemistry and Bioinformatics*. Vol. 3, No. 1, January 2013.

Jaskula, B., Kafarski, P., Aerts, G., Cooman, L. C. (2008). A Kinetic Study on the Isomerization of Hop - Acids. *Journal of agricultural and food chemistry*. 56, 5408-6415.

Kappler, S., Krahl, M., Geissinger, C., Becker, T., Krottenthaler, M. (2010). Degradation of Iso-α-Acids During Wort Boiling. *Journal of The Institute of Brewing.* 116 (4), 332-338

Kostrzewa, D., Inger, D. A., Rój, E., Grzeda, K., Kozlowski, K. (2016*). Isomerization of hop extract α-acids. Institute of brewing and distilling.*

Kreisz, S. (2009). Malting. In Eßlinger, G. M. (Ed). *Handbook of brewing: Processes, technology, markets.* (pp 147-164). Wiley-VCH.

Krottenthaler, M., Back, W. & Zarnkow, M. (2009). Wort production. In Eßlinger, G. M. (Ed). *Handbook of brewing: Processes, technology, markets.* (pp 165-202). Wiley-VCH.

Kubule, A., Zogla, L., Ikaunieks, J. & Rosa, M. (2016). Highlights on energy efficiency improvements: a case of a small brewery. *Journal of Cleaner Production*. 138, 275-286.

Kunimune, T., Shellhammer, H. (2008). Foam-Stabilizing Effects and Cling Formation Patterns of Iso-r-acids and Reduced Iso-r-acids in Lager Beer. *Journal of agricultural and food chemistry*. 56, 8629-8634.

Kunze, W. (2004a). Beer - the Oldest Drink for the Common Man. In Technology Brewing and Malting (3rd ed., pp. 19–31). VLB Berlin

Kunze, W. (2004b). Malt production. In Technology Brewing and Malting (3rd ed., pp. 97-187). VLB Berlin.

Kunze, W. (2004c). Wort production. In Technology Brewing and Malting (3rd ed., pp. 195-359). VLB Berlin.

Kunze, W. (2004d). Beer production. In Technology Brewing and Malting (3rd ed., pp. 367-525). VLB Berlin.

Kunze, W. (2004e). Feeling the beer. In Technology Brewing and Malting (3rd ed., pp. 532-712). VLB Berlin.

Leiper, K. A. & Miedl, M. (2006). Brewhouse technology. In Priest, F. G. & Stewart, G. G. (Eds). *Handbook of Brewing.* 2nd ed. pp 385-444). Taylor & Francis group.

Lindemann (2009). Filtration and stabilization. In Eßlinger, G. M. (Ed). *Handbook of brewing: Processes, technology, markets.* (pp 225-255). Wiley-VCH.

Malowicki, M. G., Shellhammer, T. H. (2005). Isomerization and degradation kinetics of hop (Humulus lupulus) acids in a model wort-boiling system. *Journal of Agricultural and Food Chemistry.* 53, 4435-4439.

Malowicki, M. G., Shellhammer, T. H. (2006) Factors Affecting Hop Bitter Acid Isomerization Kinetics in a Model Wort Boiling System, *Journal of the American Society of Brewing Chemists*, 64:1, 29-32

Meneguzzo, F., Zabini, F., Albanese, L. & Crisci, A. (2019). Novel affordable, reliable and efficient technologies to help addressing the water-energy-food nexus. *European Journal of Sustainable Development*. 8,4, 1-17.

Michael Smith engineers Ltd. (2022). *Centrifugal Pump Selection*. https://columbiacollege-ca.libguides.com/apa/websites

Michel, F. C. J. & Kozyuk, O. (2015). Hydrodynamic cavitation processing. In Letcher, T. M., Scott, J. L. & Patterson, D. A. (Eds). *Chemical processes for a sustainable future.* (pp. 84-142) Royal society of chemistry.

Munroe, J. H. (2006). Fermentation. In Priest, F. G. & Stewart, G. G. (Eds). *Handbook of Brewing. (*2nd ed. pp 448-521). Taylor & Francis group.

Nilesh, P. V., Gogate, P. R. & Pandit, A. B. (2000). Optimization of hydrodynamic cavitation using a model Reaction. *Chemical Engineering and Technology.* 23(5), 683-890.

Portaria nº 91/2022, de 9 de fevereiro da Economia e Transição Digital e Agricultura. (2022). Diário da República: I série, nº 28. *https://dre.pt/dre/detalhe/portaria/1-1996-621333*

Rhoades, J. (2021, June 21st). *Backyard hops plant: how to plant hops and hops plant history*. Gardening Know Know. https://www.gardeningknowhow.com/edible/vegetables/hops/growing-hops-plants.htm

Russel, I. (2018). Yeast. In Stewart, G. G., Russel, I., Anstruther, A. (Eds). *Handbook of Brewing.* (3rd ed. pp 226-248. Taylor & Francis group.

Shah, Y.T., Pandit, A.B. Pandit & Moholkar V. S. (1999). Sources and types of cavitation. In *Cavitation Reaction Engineering*. (pp. 1-14). Springer.

Slawitsch, B. M., Weiss, W., Schnitzer, H. & Brunner, C. (2011). The green brewery concept e Energy efficiency and the use of renewable energy sources in breweries. Applied Thermal Engineering. 31 (2011), 2123-2134

Steenackers, B., Cooman, L. D., Vos, D. D. (2015). Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: A review. *Food Chemistry.* 172, 742-756.

Terentiev, A. G., Kirschner, I. N. & Uhlman, J. S. (2011). A physical description of cavitation. In The Hydrodynamics of Cavitation Flows. (pp.11-26). Backbone publishing company.

Thanekar, P. & Gogate, P. (2018). Application of hydrodynamic cavitation reactors for treatment of wastewater containing organic pollutants: intensification using hybrid approaches. Fluids. 3,98, 1-24.

The brewers of Europe. (2021). *European beer trends statistics report. https://brewersofeurope.org/uploads/mycms-files/documents/publications/2021/european-beer-statistics-2020.pdf*

Universidade do Minho. (2019). Capítulo III – Equipamento para o transporte de Fluídos. *UC Fenómenos de Transferência I.*

Universidade do Minho. (2019). Capítulo III – Equipamento para o transporte de Fluídos. *UC Fenómenos de Transferência I.*

Universidade do Minho. (2020). EB#04 – A produção de cerveja. *UC Engenharia Bioquímica.*

Willaert, R. (2007). The beer brewing Process: Wort production and beer fermentation. In Hui, Y. H. (Ed). *Handbook of food products manufacturing*. (pp. 443-507). John Wiley & Sons, Inc.

Wunderlich, S. & W. Back (2009). Beer making, hops and yeast. In Preedy, V. R. (Ed). *Beer in Health and Disease Prevention*. (pp. 1-113) Academic Press.

Zheng, H. (2021). *Experimental study and characterization of bubble behaviors in the orifice-induced hydrodynamic cavitation*. Electronic Thesis and Dissertation Repository. 8131. https://ir.lib.uwo.ca/etd/8131

APPENDICES

Appendix A - Tables

A.1 – Vapor pressure of pure water

Table A.I indicates the values of the vapor pressure of water for their respective temperatures.

Table A. I - Vapor pressure of water according to their temperatures

Temperature (°C)	*Vapor pressure (Pa)*
20	2313.30
30	4219.83
40	7358.73
50	12327.51
60	19922.03
70	31171.34
80	47370.95
90	70113.59
100	101315.91

A.2 Degree Plato

Table A.II indicates the values of the density of the beer in order to achieve their respective degree Plato.

Table A. II - Correlation between density and the degree Plato of *beer*

Degrees Plato	*Density [Kg/dm³]*	*Degrees Plato*	*Density [Kg/dm³]*
8	1.032	**17**	1.070
9	1.036	**18**	1.074
10	1.040	**19**	1.079
11	1.044	**20**	1.083
12	1.048	**21**	1.087
13	1.053	**22**	1.092
14	1.057	**23**	1.096
15	1.061	**24**	1.101
16	1.065	**25**	1.106

Appendix B – Auxiliary Calculations

B.1 – Calculation of the alpha acids content of the sample

In order to calculate the concentration of alpha acids in each sample, the next equation is applied:

$$Ca = Aa \times fa \tag{6}$$

From the previous equation, Aa indicates the peak area given by the HPLC method for the alpha acids content. To convert those areas to the respective concentrations it is multiplied by a conversion factor fa for each alpha acid (co-humulone, n+ad-humulone) which is given by the next equation.

$$fa = \frac{\frac{m_{ICE-4} \times C_{ICE-4}}{5} \times \frac{10000}{100}}{A} \tag{7}$$

From the previous equation, m_{ICE-4} is the weight of ICE-4 standard (g/100 ml) and C_{ICE-4} is the standard yield (%) of each alpha acid. The multiplication of those values is divided by five because of the dilution of the calibration solution. The standard peak area given for each alpha acid is represented by A, and the last part of the equation are adjustments to the required units.

Calculation example for the experiment with 90 °C with 0,1 cavitation number:

For this experiment:

- m_{ICE-4} = 0.5683 g/l
- C_{ICE-4} for co-humulone = 10.98 %
- C_{ICE-4} for n+ad-humulone = 31.6 %

The standard peak areas for each alpha acid are represented in the next Table B.I. The number used for the equation is the average of each alpha acid concentration

Table B. I - Standard peak values given for alpha acids

B1(co-humumole)	*B2 (n+ad-humulone)*
1500.881	4308.459
1497.739	4299.701

- Average B1 = 1499.310
- Average B2 = 4304.080

The first step is to calculate the conversion factor, fa. The conversion factor for co-humulone concentration is given by:

$$fa = \frac{\frac{0.5683 \times 10.98}{5} \times \frac{1000 \times 10}{100}}{1499.310} = 0.0832$$

The conversion factor for n+ad-humulone concentration is given by:

$$fa = \frac{\frac{0.5683 \times 31.6}{5} \times \frac{1000 \times 10}{100}}{4304.080} = 0.0834$$

Finally, applying Equation 6, it ispossible to determine the concentration of alpha acids for each sample. For the sample 1, the peak area of co-humulone obtained through HPLC, was 134.552 and for n+ad-humulone was 215.393. Knowing this, it is possible to calculate the concentrations of each alpha acid. Its summatory gives the total concentration of alpha acids for sample 1. The procedure for the remaining samples is the same.

$Ca_{co-humulone} = 134.552 \times 0.832$ = 11.19 mg/L

$Ca_{n+ad-humulone} = 215.393 \times 0.834$= 17.96 mg/L

$Ca_{total} = 11.19 + 17.96 = 29.16$ mg/L

B.2 - Calculation of the iso-alpha acids content of the sample

In order to calculate the concentration of iso-alpha acids of each sample of one experiment, the procedure is very close to the one presented in the previous topic A.1. The only difference are the formulas, which have small variations.

In order to calculate the concentration of iso-alpha acids in each sample, the next equation is applied:

$$Ciso - a = Aiso \times fiso \tag{8}$$

From the previous equation, $Aiso$ indicates the peak area given by the HPLC method for the alpha acids content. To convert those areas to the respective concentrations it is multiplied by a conversion factor $fiso$. Contrary to the conversion factor of the alpha acids, this one is the same for all the iso-alpha acids, because C_{ICS-14} is the same for all of them (in the previous one C_{ICE-4} was different for each alpha acid).

$$fiso = \frac{m_{ICS-14} \times C_{ICS-14}}{A} \times \frac{10000}{100} \tag{9}$$

From the previous equation 7, the term changed to m_{ICS-14}, since now corresponds to the weight of the ICS-14 standard (g/100ml).

B.3 – Calculation of the isomerization

The formula to calculate the isomerization is given by Equation 10. Since the concentration of alpha acids is not totally quantified due to is low solubilization, the formula used to calculate the isomerization has as its denominator the summatory between the alphas and iso-alpha acids from the sample with highest number. In the numerator it is subtracted the final amount of iso-alpha acids by the amount present in the first sample, due to isomerization does not take place at 0 min.

$$Isomerization\ (\%) = \frac{C_{iso}\ last\ sample - \ C_{iso}\ first\ sample}{\Sigma\ C_{iso} + \ C_a\ sample\ with\ highest\ number}$$

(10)

Where C_{iso} indicates the concentration of iso-alphas and C_a, the concentration of alpha-acids.

Appendix C – Experimental data

C.1 – Experiment at 100 °C without hydrodynamic cavitation

Figure C.I, presents the variation of alpha acids during the 90 min.

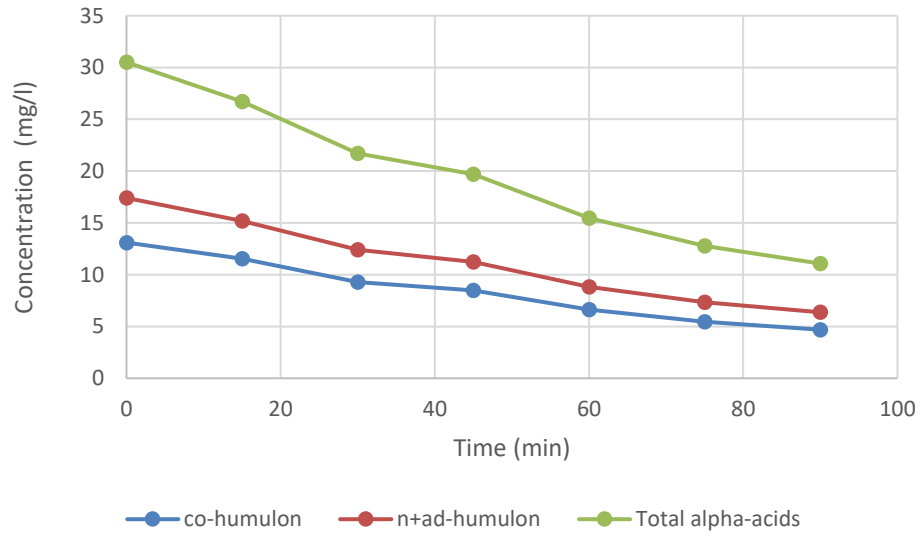


Figure C. I - Variation of alpha acid homologs during the 90 min of experience (100 °C without HC).

Figure C.II, presents the variation of iso-alpha acids during the 90 min.

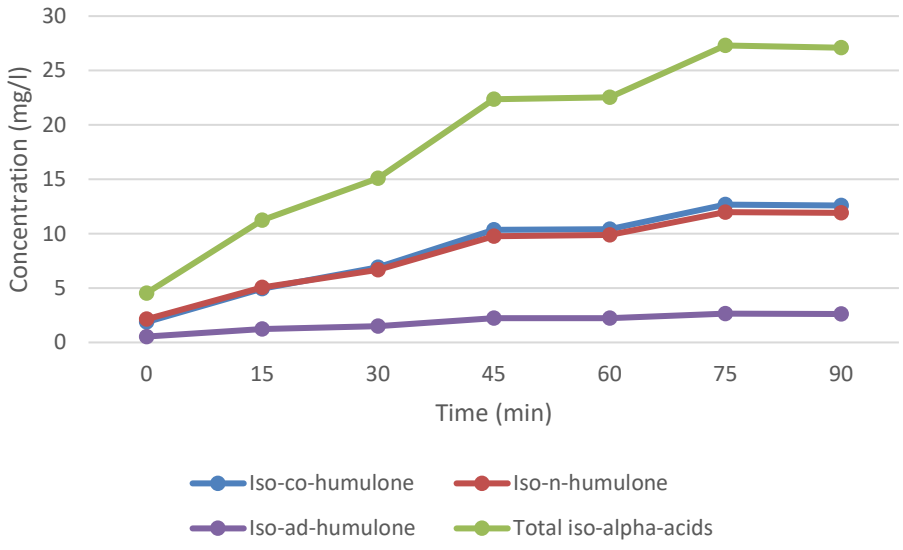


Figure C. II - Variation of iso-alpha acid homologs during the 90 min of experience (100 °C without HC).

C.2 - Experiment at 70 °C with 0.062 cavitation number

Table C.I shows the concentrations obtained during 90 min of the experiment

Table C. I - Data relative to the wort boiling experiment at 70 °C with 0.062 CN

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.50	0.59	0.19	1.28	13.31	19.06	32.37	33.64
15	0.81	1.04	0.30	2.15	14.58	21.40	35.98	38.13
30	1.12	1.47	0.37	2.96	13.89	19.87	33.76	36.72
45	1.37	2.04	0.62	4.03	13.67	19.67	33.34	37.38
60	1.90	2.80	0.86	5.57	13.86	19.72	33.57	39.14
75	1.98	2.93	0.89	5.79	12.78	17.78	30.57	36.36
90	2.53	3.79	1.10	7.42	12.71	17.43	30.14	37.56

Figure C.IV, presents the variation of alpha acids during the 90 min.

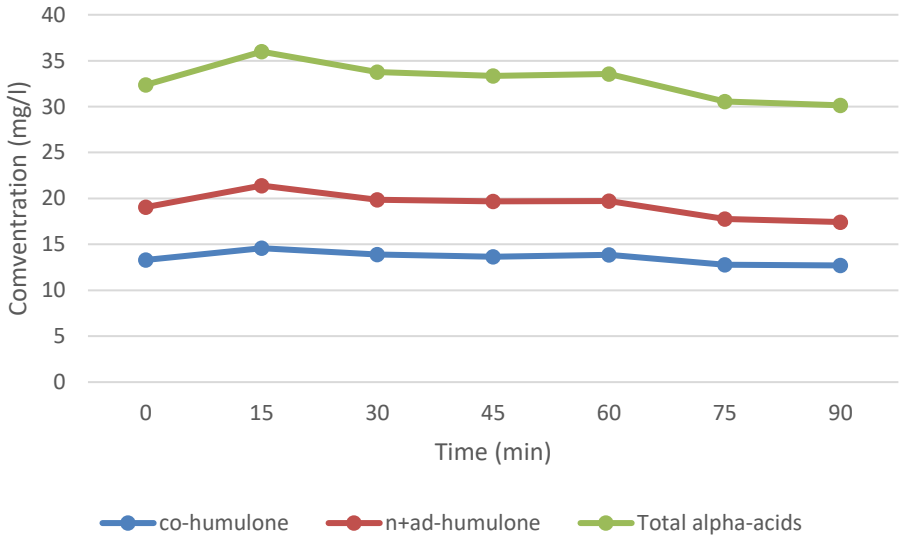


Figure C. IV - Variation of alpha acid homologues during the 90 min of experience (70 °C with 0.062 CN).

Figure C.V, presents the variation of iso-alpha acids during the 90 min.

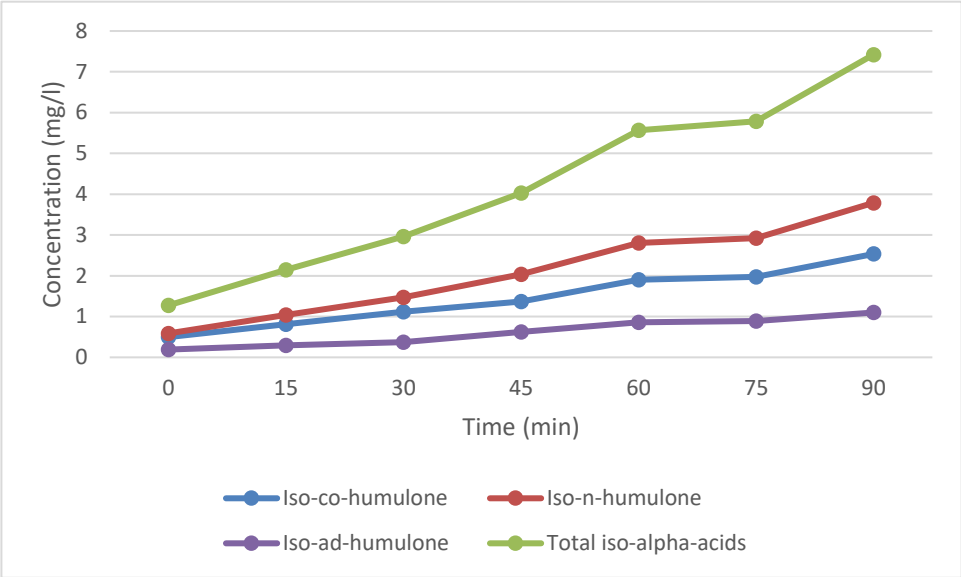


Figure C. V - Variation of iso-alpha acid homologues during the 90 min of experience (70 °C with 0.062 CN).

C.3 - Experiment at 70 °C with 0.1 cavitation number

Figure C.VI, presents the variation of alpha acids during the 90 min.

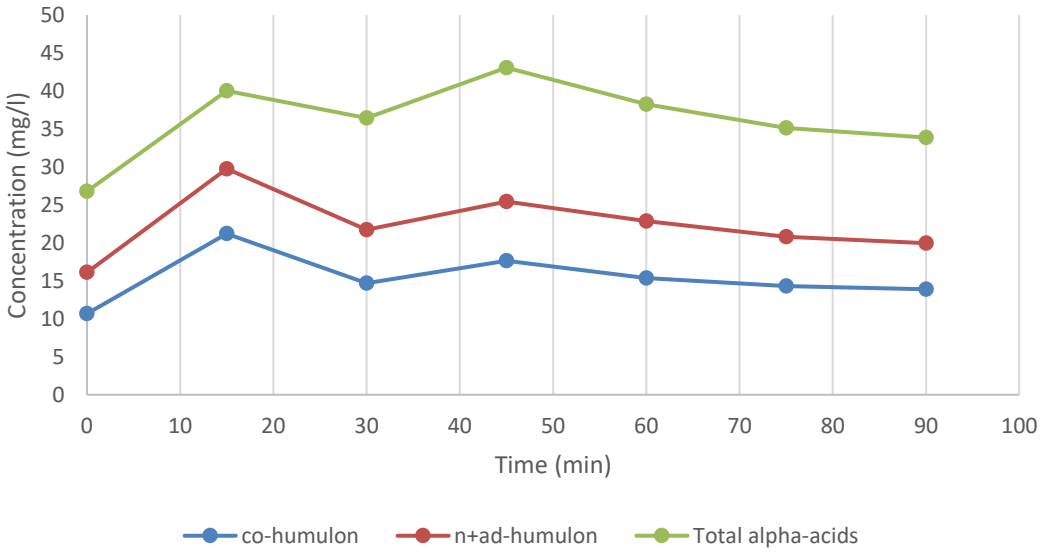


Figure C. VI - Variation of alpha acid homologues during the 90 min of experience (70 °C with 0.1 CN).

Figure C.VII, presents the variation of iso-alpha acids during the 90 min.

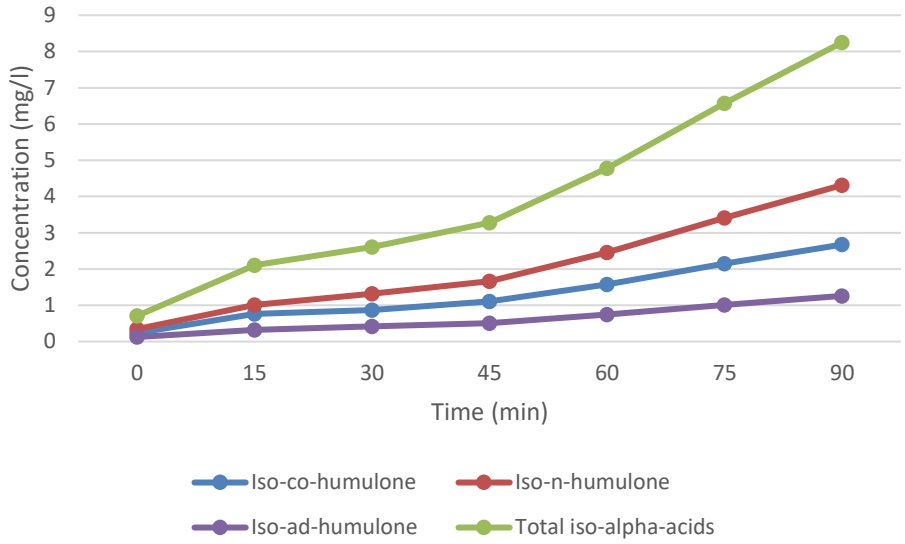


Figure C. VII - Variation of iso-alpha acid homologues during the 90 min of experience (70 °C with 0.1 CN).

C.4 - Experiment at 70 °C with 0.15 cavitation number (with extract)

Table C.II, shows the concentrations obtained during 90 min of the experiment.

Table C. II - Data relative to the wort boiling experiment at 70 °C with 0.15 CN (using hop extract)

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.55	0.79	0.29	1.64	16.40	25.52	41.92	43.56
15	0.74	1.04	0.35	2.13	14.38	21.74	36.13	38.26
30	1.04	1.52	0.47	3.03	15.26	23.48	38.75	41.78
45	1.23	1.87	0.56	3.67	13.67	19.87	33.54	37.21
60	1.78	2.81	0.82	5.41	14.06	21.15	35.22	40.63
75	1.64	2.56	0.75	4.95	13.13	19.39	32.52	37.47
90	2.13	3.30	0.98	6.40	14.32	21.27	35.59	42.00

Figure C. VIII, presents the variation of alpha acids during the 90 min.

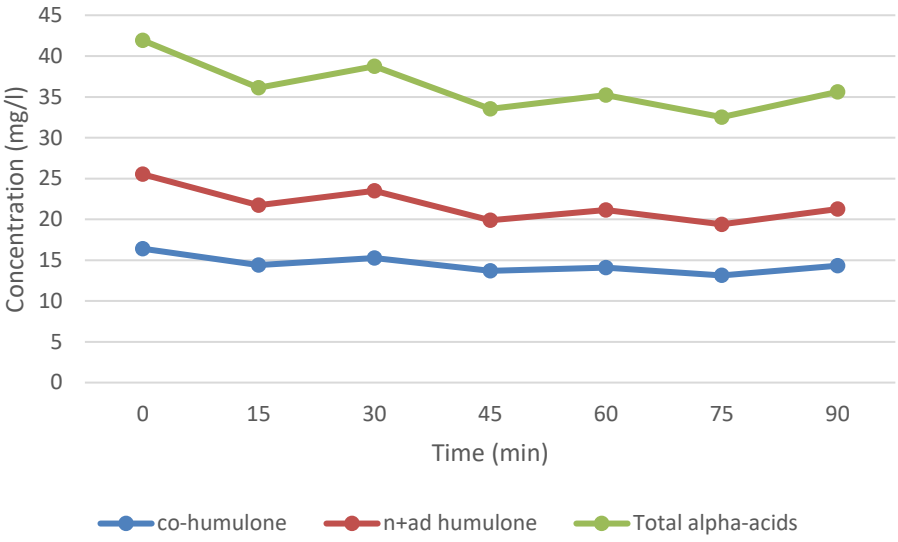


Figure C. VIII - Variation of alpha acid homologues during the 90 min of experience using hop extract (70 °C with 0.15 CN).

Figure C.IX, presents the variation of iso-alpha acids during the 90 min.

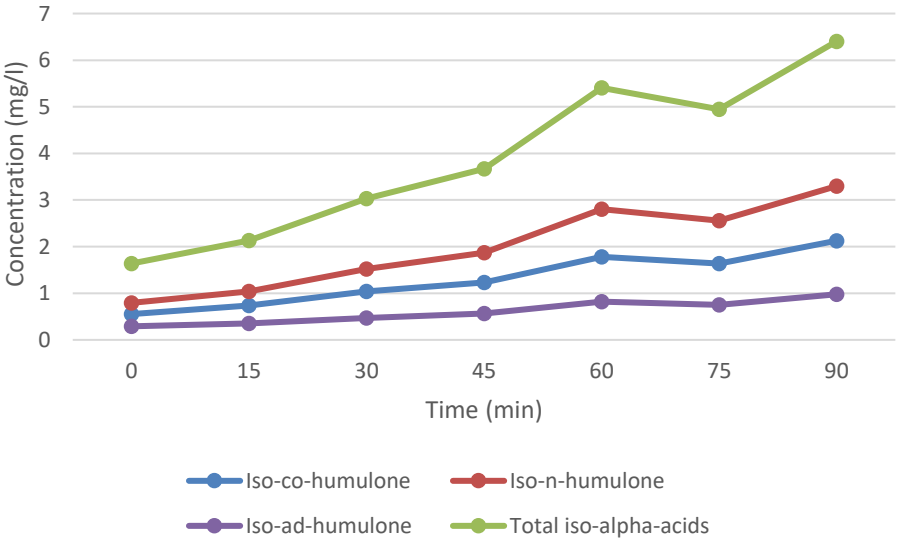


Figure C. IX - Variation of iso-alpha acid homologues during the 90 min of experience using hop extract (70 °C with 0.15 CN).

C.5 - Experiment at 70 °C with 0.15 cavitation number (with pellets)

Table C.III, shows the concentrations obtained during 90 min of the experiment.

Table C. III - Data relative to the experiment at 70 °C with 0.15 CN (using hop pellets)

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.43	0.23	0.13	0.79	4.93	13.24	18.17	18.96
15	0.66	0.51	0.22	1.39	8.19	22.16	30.36	31.75
30	0.86	0.90	0.30	2.06	8.53	24.62	33.16	35.22
45	0.93	1.24	0.39	2.56	7.97	23.12	31.10	33.66
60	0.91	1.51	0.44	2.87	7.32	21.02	28.35	31.22
75	1.13	1.89	0.53	3.55	7.23	20.05	27.29	30.84
90	0.95	2.50	0.66	4.12	7.35	20.10	27.45	31.57

Figure C.X, presents the variation of alpha acids during the 90 min.

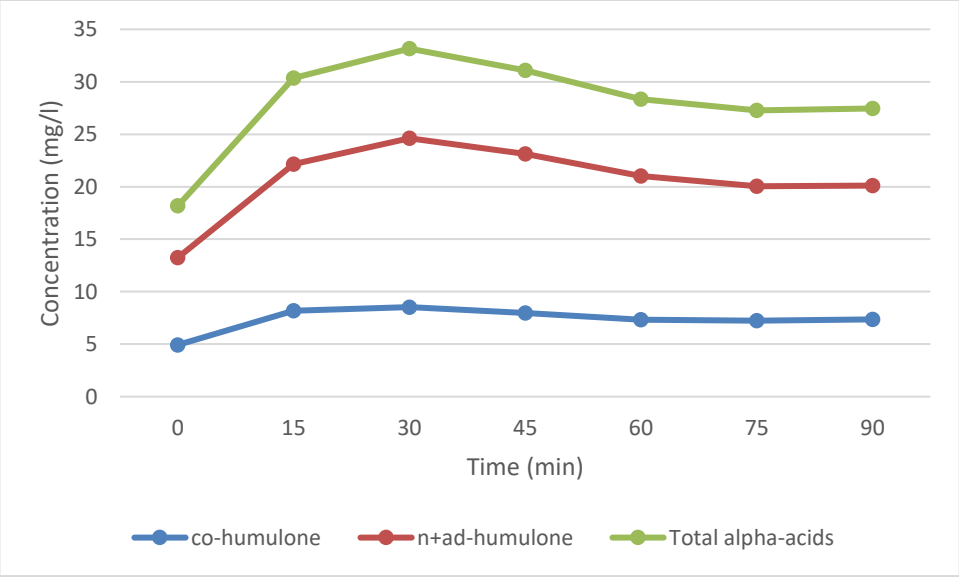


Figure C. X - Variation of alpha acid homologues during the 90 min of experiment using hop pellets (70 °C with 0.15 CN).

Figure C.XI, presents the variation of iso-alpha acids during the 90 min.

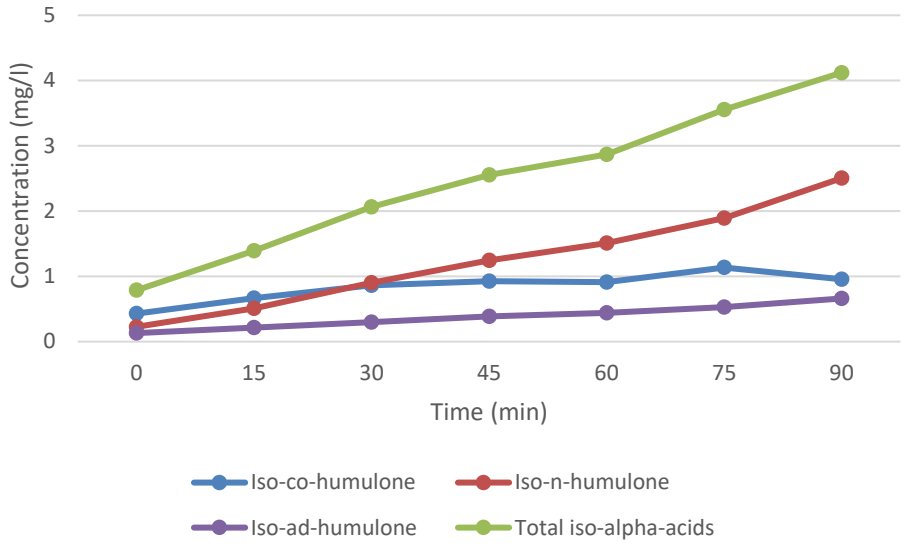


Figure C. XI - Variation of iso-alpha acid homologues during the 90 min of experiment using hop pellets (70 °C with 0.15 CN).

C.6 - Experiment at 80 °C with 0.062 cavitation number

Table C.IV, shows the concentrations obtained during 90 min of the experiment.

Table C. IV - Data relative to the experiment at 80 °C with 0.062 CN

Sample (min)	***iso co-humulone***	***iso n-humulone***	***n+ad-humulone***	***Σ Iso-Alpha acids***	***co-humulone***	***n+ad-humulone***	***Σ Alpha-acids (mg/L)***	***Alpha + Iso-alpha acids (mg/L)***
0	1.09	1.03	0.31	2.44	12.09	16.80	28.89	31.33
15	1.95	2.45	0.72	5.12	14.01	20.40	34.40	39.52
30	2.55	3.52	1.02	7.09	12.98	18.72	31.69	38.78
45	3.47	4.98	1.44	9.90	12.60	18.05	30.64	40.54
60	3.78	5.55	1.58	10.91	11.29	15.91	27.19	38.10
75	5.03	7.46	2.13	14.62	11.29	15.42	26.70	41.32
90	6.06	8.88	2.50	17.44	10.58	13.80	24.37	41.81

Figure C.XII, presents the variation of alpha acids during the 90 min.

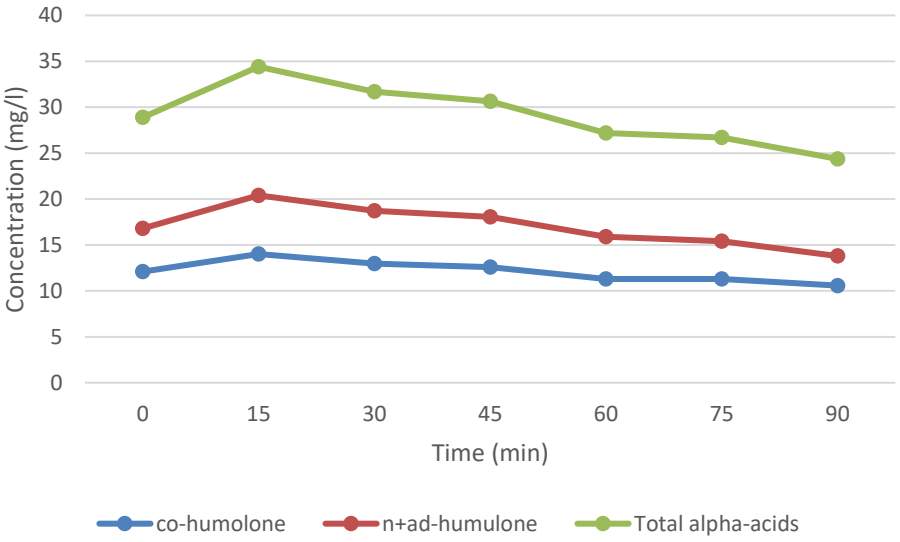


Figure C. XII - Variation of alpha acid homologues during the 90 min of experiment using hop pellets (80 °C with 0.062 CN).

Figure C.XIII, presents the variation of iso-alpha acids during the 90 min.

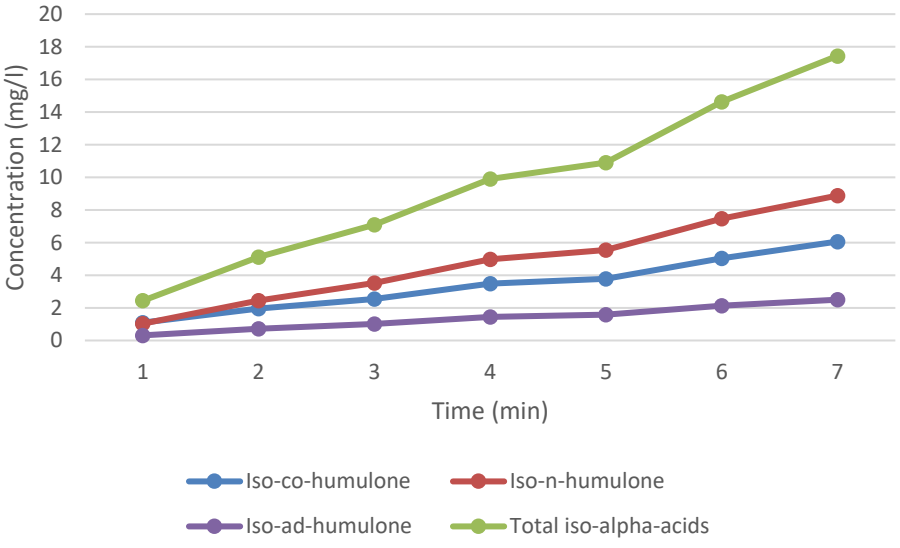


Figure C. XIII - Variation of iso-alpha acid homologues during the 90 min of experiment (80 °C with 0.062 CN).

C.7 - Experiment with 80 °C with 0.1 cavitation number

C.7.1. Experiment I

Table C.V, shows the concentrations obtained during 90 min of the experiment.

Table C. V - Data relative to the experiment at 80 °C with 0.1 (Experiment I)

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.54	0.83	0.37	1.74	12.07	18.41	30.48	32.22
15	1.26	1.92	0.63	3.81	13.28	19.76	33.04	36.85
30	2.06	3.14	0.97	6.16	12.80	19.38	32.18	38.34
45	2.95	4.44	1.33	8.72	12.12	17.79	29.91	38.62
60	3.77	5.72	1.65	11.14	12.19	17.60	29.78	40.93
75	4.66	7.09	2.04	13.80	11.64	16.38	28.02	41.82
90	5.93	9.07	2.53	17.53	11.55	15.59	27.14	44.66

Figure XIV, presents the variation of alpha acids during the 90 min.

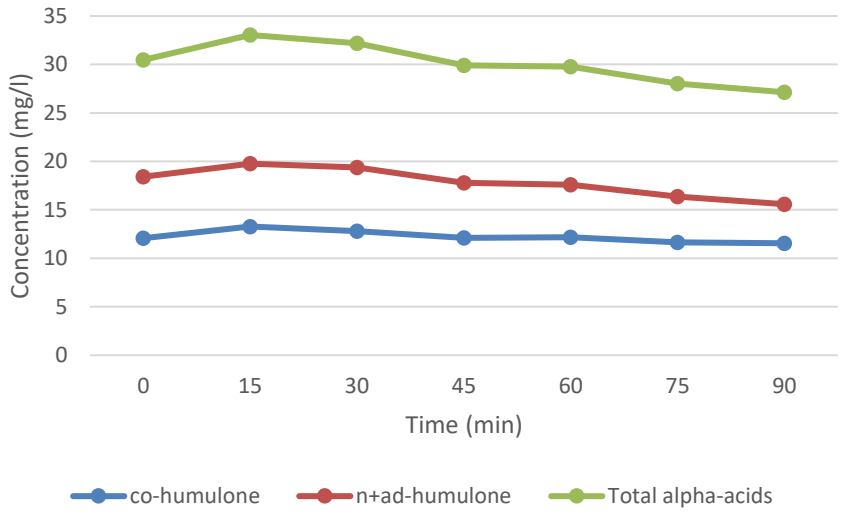


Figure C. XIV - Variation of alpha acid homologues during the 90 min of experiment I (80 °C with 0.1 CN).

Figure C.XV, presents the variation of iso-alpha acids during the 90 min.

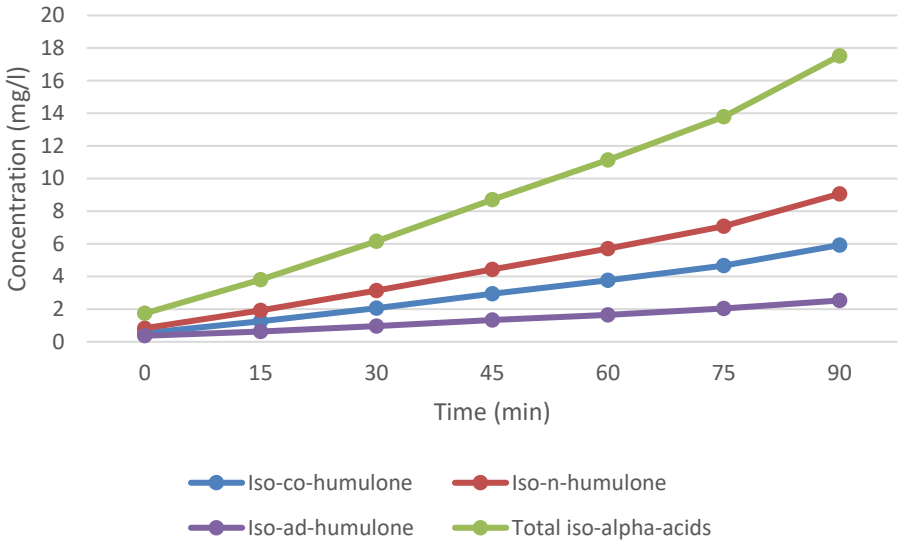


Figure C. XV - - Variation of iso-alpha acid homologues during the 90 min of experiment I (80 °C with 0.1 CN).

C.7.2. Experiment II

Table C.V, shows the concentrations obtained during 90 min of the experiment.

Table C. VI - Data relative to the experiment at 80 °C with 0.1 (Experiement II)

Sample (min)	***iso co-humulone***	***iso n-humulone***	***n+ad-humulone***	***Σ Iso-Alpha acids***	***co-humulone***	***n+ad-humulone***	***Σ Alpha-acids (mg/L)***	***Alpha + Iso-alpha acids (mg/L)***
0	1.12	1.09	0.33	2.54	13.00	19.55	32.53	35.07
15	1.95	2.49	0.75	5.19	13.24	19.01	32.24	37.43
30	2.63	3.70	1.05	7.37	11.94	17.10	29.04	36.41
45	3.69	5.30	1.51	10.50	12.04	16.60	28.64	39.14
60	4.46	6.47	1.78	12.71	11.14	14.94	26.07	38.78
75	5.75	8.63	2.31	16.69	10.76	14.12	24.88	41.56
90	6.78	10.28	2.83	19.89	9.94	12.68	22.61	42.50

Figure C.XVI, shows the variation of alpha acids during the 90 min.

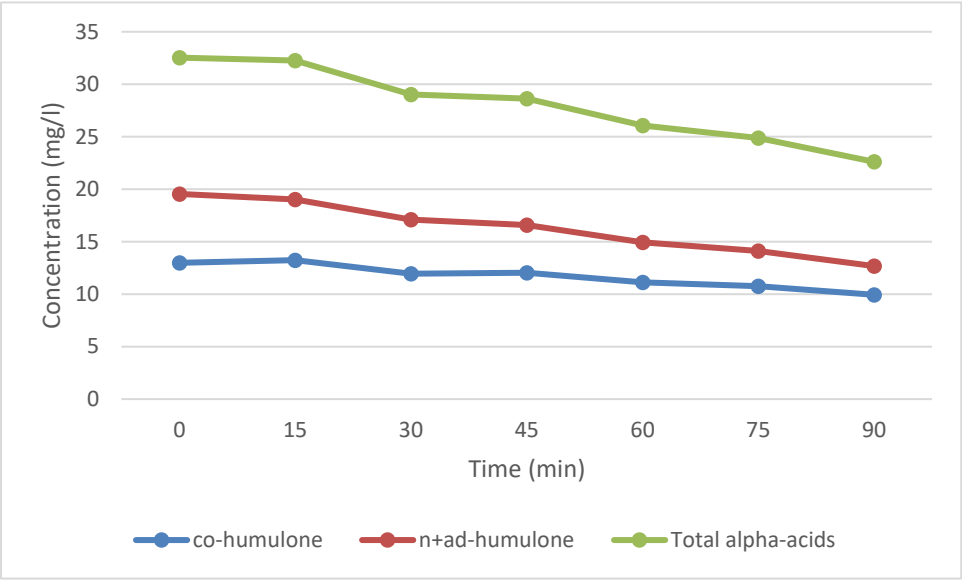


Figure C. XVI - Variation of alpha acid homologues during the 90 min of experiment II (80 °C with 0.1 CN)

Figure C.XVII, shows the variation of iso-alpha acids during the 90 min.

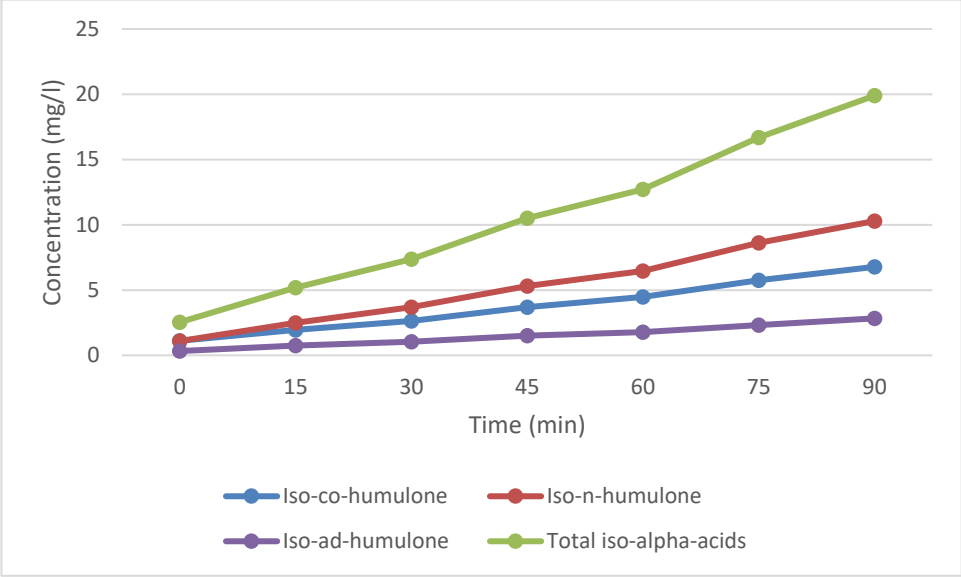


Figure C. XVII - Variation of iso-alpha acid homologues during the 90 min of experiment II (80 °C with 0.1 CN).

C.8 - Experiment at 80 °C at 0.15 cavitation number

Table C.VII, shows the concentrations obtained during 90 min of the experiment.

Table C. VII - Data relative to the experiment at 80 °C with 0.15

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.85	0.98	0.29	2.12	9.56	14.45	24.00	26.11
15	1.86	2.45	0.71	5.01	11.93	18.19	30.12	35.13
30	2.34	3.25	0.94	6.52	11.14	16.96	28.09	34.62
45	2.73	4.05	1.13	7.91	10.26	15.45	25.70	33.61
60	3.69	5.57	1.57	10.82	9.92	14.49	24.40	35.23
75	4.50	6.91	1.93	13.35	9.38	13.29	22.66	36.01
90	5.31	8.21	2.30	15.82	8.84	12.33	21.17	36.98

Figure C. XVII, presents the variation of alpha acids during the 90 min.

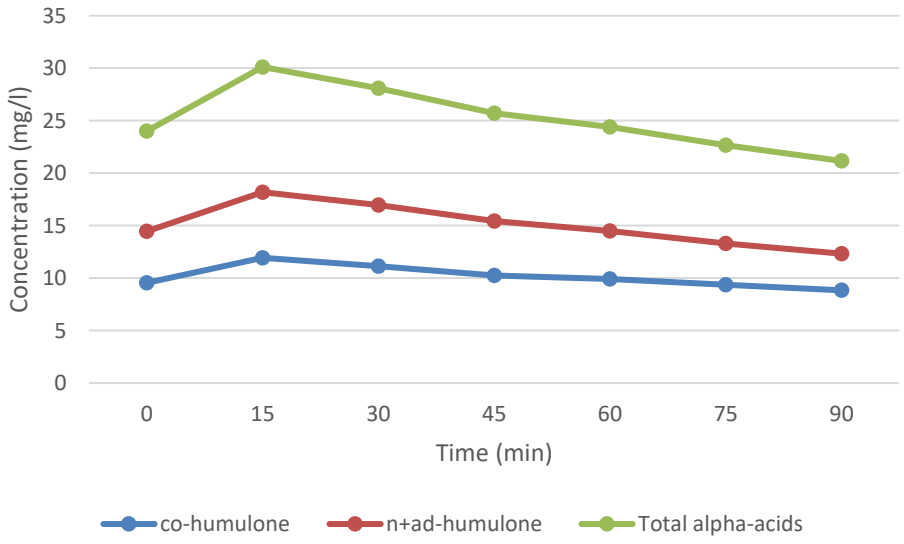


Figure C. XVIII - Variation of alpha acid homologues during the 90 min (80 °C with 0.15 CN).

Figure C. XIX, presents the variation of iso-alpha acids during the 90 min.

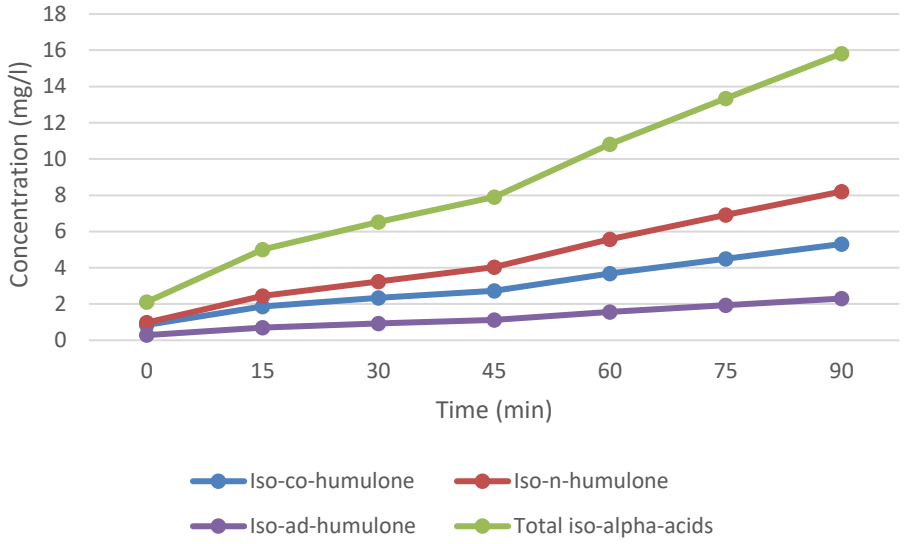


Figure C. XIX - Variation of alpha acid homologues during the 90 min (80 °C with 0.15 CN).

C.9 – Experiment at 90 °C with 0.062 cavitation number

Table C. VIII, shows the concentrations obtained during 90 min of the experiment.

Table C. VIII - Data relative to the experiment at 90 °C with 0.062

Sample (min)	***iso co-humulone***	***iso n-humulone***	***n+ad-humulone***	***Σ Iso-Alpha acids***	***co-humulone***	***n+ad-humulone***	***Σ Alpha-acids (mg/L)***	***Alpha + Iso-alpha acids (mg/L)***
0	0.76	1.05	0.37	2.18	10.91	16.00	26.91	29.09
15	2.35	3.26	0.98	6.59	12.00	17.04	29.04	35.64
30	4.40	6.33	1.83	12.56	10.27	14.61	24.88	37.44
45	5.11	7.26	2.05	14.42	8.38	11.64	20.03	34.45
60	7.55	10.86	3.00	21.42	8.03	10.84	18.86	40.28
75	6.77	9.67	2.61	19.05	5.64	7.18	12.81	31.87
90	10.54	14.78	3.94	29.26	6.13	7.58	13.71	42.97

Figure C. XX, presents the variation of alpha acids during the 90 min.

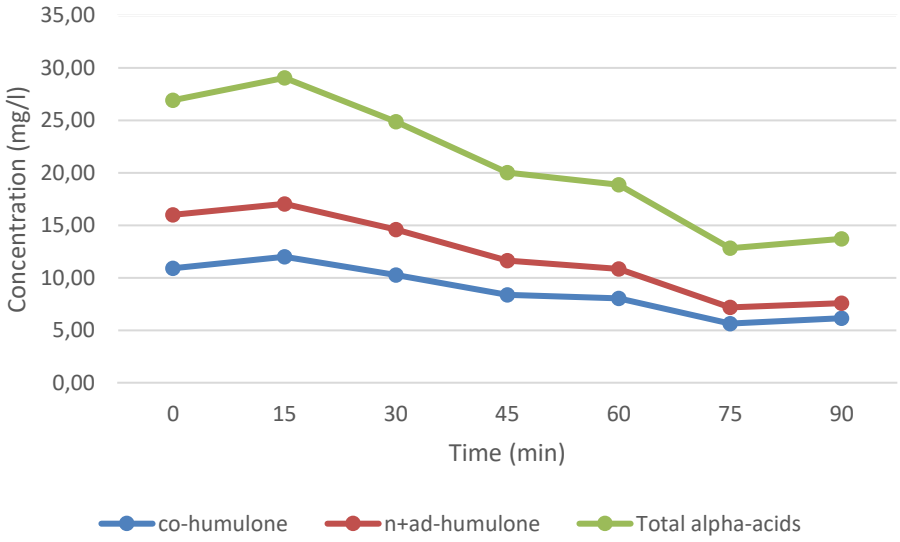


Figure C. XX - Variation of iso-alpha acid homologues during the 90 min (90 °C with 0.062 CN).

Figure C. XXI, presents the variation of iso-alpha acids during the 90 min.

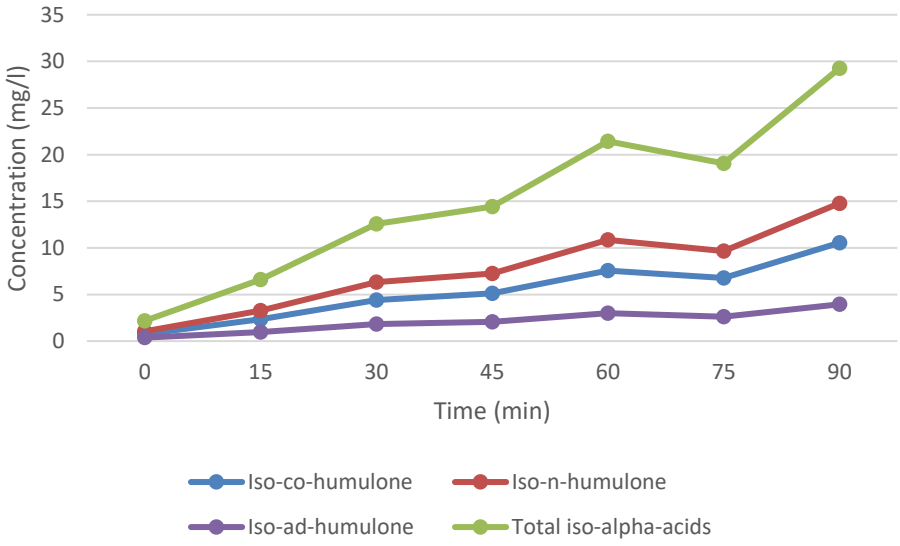


Figure C. XXI - Variation of alpha acid homologues during the 90 min (90 °C with 0.062 CN).

C.10 - Experiment at 90 °C with 0.1 cavitation number

Table C. IX, shows the concentrations obtained during 90 min of the experiment.

Table C. IX - Data relative to the experiment at 90 °C with 0.1

Sample (min)	*iso co-humulone*	*iso n-humulone*	*n+ad-humulone*	*Σ Iso-Alpha acids*	*co-humulone*	*n+ad-humulone*	*Σ Alpha-acids (mg/L)*	*Alpha + Iso-alpha acids (mg/L)*
0	0.97	1.37	0.35	2.69	11.20	18.01	29.20	31.90
15	2.95	4.05	1.14	8.14	9.67	14.76	24.42	32.56
30	4.94	6.94	1.98	13.86	9.26	13.73	22.99	36.85
45	5.69	8.04	2.24	15.98	7.78	11.14	18.92	34.90
60	6.88	9.77	2.72	19.38	6.97	9.91	16.87	36.25
75	7.28	10.49	2.85	20.62	6.59	8.73	15.32	35.94
90	9.66	13.03	3.63	26.72	6.34	8.46	14.76	41.48

Figure C. XXII, presents the variation of alpha acids during the 90 min.

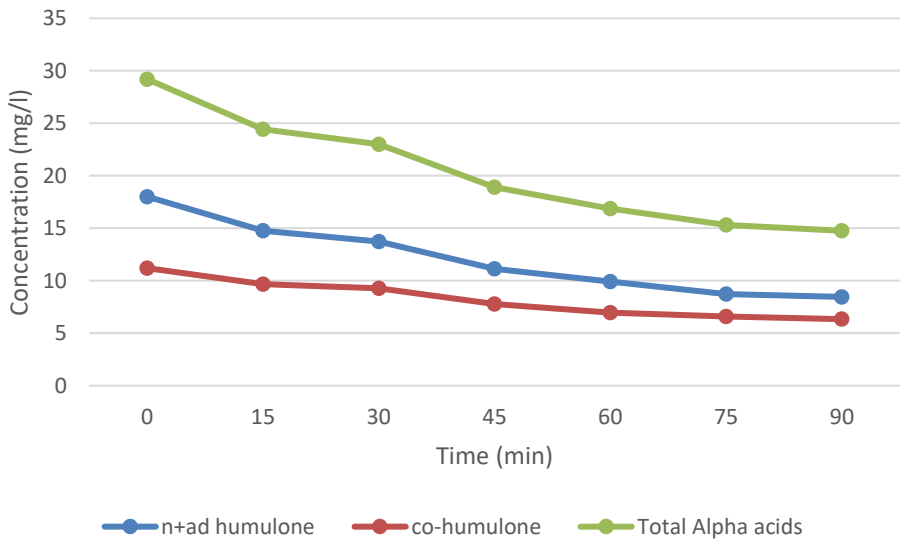


Figure C. XXII - Variation of iso-alpha acid homologues during the 90 min (90 °C with 0.1 CN).

Figure C. XXIII, presents the variation of iso-alpha acids during the 90 min.

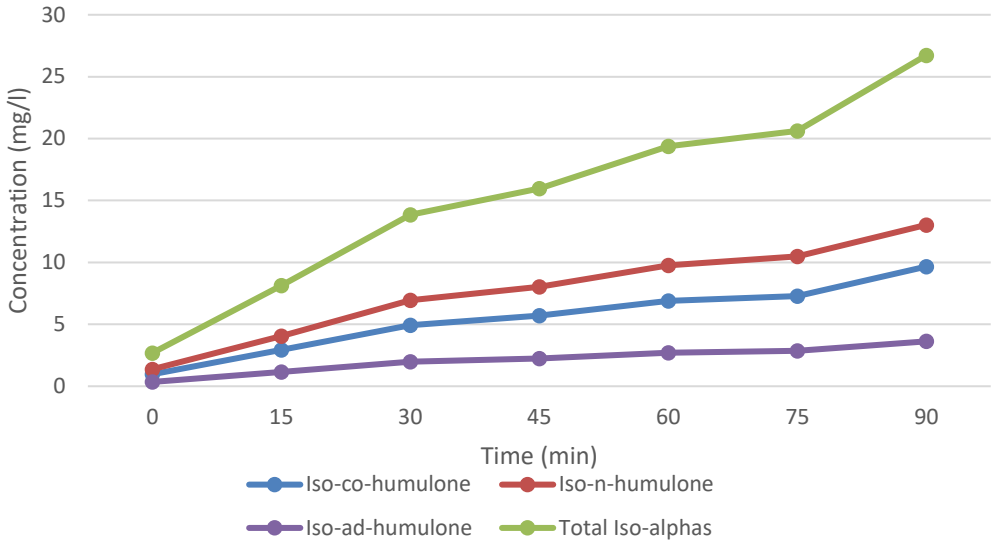


Figure C. XXIII - Variation of iso-alpha acid homologues during the 90 min (90 °C with 0.1 CN).

C.11 - Experiment with 90 °C at 0.15 cavitation number

Table C. X shows the concentrations obtained during 90 min of the experiment.

Table C. X - Data relative to the experiment at 90 °C with 0.15

Sample (min)	***iso co-humulone***	***iso n-humulone***	***n+ad-humulone***	***Σ Iso-Alpha acids***	***co-humulone***	***n+ad-humulone***	***Σ Alpha-acids (mg/L)***	***Alpha + Iso-alpha acids (mg/L)***
0	4.28	4.05	1.12	9.45	16.51	26.24	42.55	51.99
15	3.24	4.06	1.14	8.44	8.96	14.34	23.19	31.63
30	5.90	7.56	2.12	15.58	9.44	14.62	23.95	39.53
45	7.07	9.33	2.57	18.97	8.20	12.31	20.42	39.39
60	7.94	10.73	2.91	21.58	7.19	10.49	17.61	39.19
75	9.47	12.93	3.47	25.86	6.75	9.58	16.28	42.14
90	10.01	13.71	3.63	27.35	5.94	7.96	13.86	41.21

Figure C. XXIV, presents the variation of alpha acids during the 90 min.

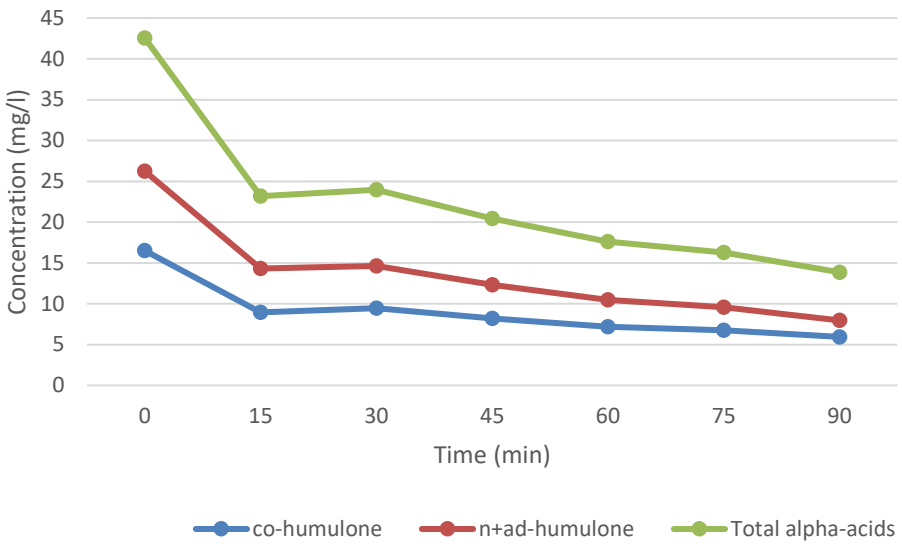


Figure C. XXIV - Variation of alpha acid homologues during the 90 min (90 °C with 0.15 CN).

Figure C. XXV, presents the variation of iso-alpha acids during the 90 min.

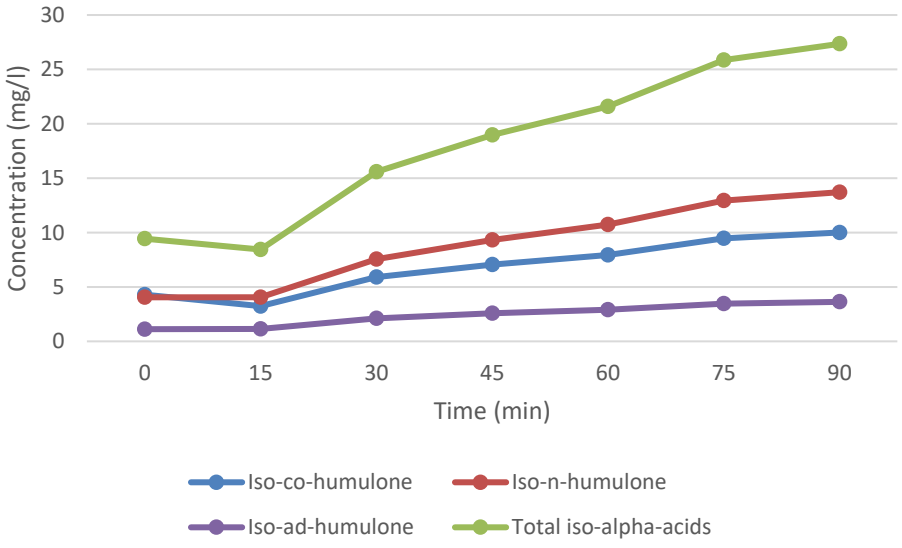


Figure C. XXV - Variation of iso-alpha acid homologues during the 90 min (90 °C with 0.15 CN).