Master's thesis

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

Christian Finnseth

Impact of high-pressure processing (HPP) and microwave (MW) treatment on selected quality and nutritional parameters of cauliflower (*Brassica oleracea* var. *Botrytis*)

Master's thesis in Food Science, Technology and Sustainability (FTMAMAT)

Supervisor: Marcin A. Kurek
Co-supervisor: Tone Mari Rode & Dagbjørn Skipnes (Nofima)

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Abstract

It is well known that a vegetable-rich diet contributes to several health benefits and a lower climate footprint. Traditional processing of vegetables such as retorting, can in many cases lower the quality of the food. In recent years, innovative food processing methods such as high-pressure processing (HPP) and microwave (MW) treatment have been shown to improve food quality. These methods have the potential of enhancing the microbial, color, texture, and nutritional quality of foods.

The purpose of this thesis was to determine the effects of high-pressure processing (HPP; 400 and 600 MPa for 2 or 5 min, 20 °C) and microwave treatment (MW; 6 kW for 90 s, followed by 1kW for 600 s) compared to a conventional retort treatment (RT; 96 °C for 45 min) of cauliflower. Microbial shelf-life (total aerobic count and spores), texture, color, drip loss, dry matter, antioxidative capacity (2,2-Diphenyl-1-picrylhydrazyl [DPPH]), total phenolic content (Folin-Ciocalteau's method), and ascorbic acid (HPLC) were analyzed before and after processing, and during storage (4°C) for up to 42 days.

Among the different treatments, HPP at 600 MPa exhibited low microbial counts between day 14 and 28 of storage, while 400 MPa had high bacterial counts already between day 7 and 14. The MW yielded high bacterial counts already at day 0 due to insufficient heating. Retorted samples remained below the detection limit (<2.3 log cfu/g) until day 42 of storage. All counts of both aerobic and anaerobic spores remained below or close to the detection limit during storage. HPP at both 400 and 600 MPa was the best method to maintain the color during storage. The texture of the cauliflower did not differ from the control during storage for HPP and MW samples, while retorted samples had a significant reduction in texture. The drip loss for HPP samples was significantly higher than the control, while MW yielded lower drip loss. For all samples, the dry matter content remained stable during storage, with few differences between treatments. The nutritional quality of cauliflower HPP at 600 MPa, 2 min remained high until day 28, while MW at day 0 increased, possibly due to enhanced extractability of nutrients. Retorted samples at day 0 resulted in 30, 27, and 40 % reduction in antioxidant activity, total phenolic content, and ascorbic acid, respectively.

The overall results of this study demonstrate that HPP and MW have the potential of preserving the quality of cauliflower compared to conventional retorting. Optimization of processing conditions for MW is needed. For both treatments, there is potential for adding synergistic treatments to further extend the shelf-life while preserving quality.

Sammendrag

Det er velkjent at en diett som er rik på grønnsaker kan gi flere helsemessige fordeler og bidra til et lavere klimafotavtrykk. Tradisjonell bearbeiding av grønnsaker slik som autoklavering, kan i mange tilfeller redusere kvaliteten på maten. De siste årene har det blitt utviklet flere innovative matprosesseringsmetoder som høytrykksbehandling (HPP) og mikrobølger (MW). Disse metodene har potensialet til å forbedre holdbarhet, farge, tekstur og ernæringskvaliteten på matvarer.

Hensikten med denne studien var å undersøke effekten av høytrykksbehandling (HPP; 400 og 600 MPa, i 2 eller 5 min, 20 °C) og mikrobølgebehandling (MW; 6 kW i 90 s, etterfulgt av 1 kW i 600 s) sammenlignet med en konvensjonell autoklavbehandling (96 °C i 45 min) på blomkål. Mikrobiell holdbarhet (totalt aerobt kimtall og sporer), tekstur, farge, drypptap, tørrstoff, antioksidativ kapasitet (2,2-difenyl-1-pikrylhydrazyl [DPPH]), totalt fenolinnhold (Folin-Ciocalteaus metode), og askorbinsyre (HPLC) ble analysert før og etter prosessering, samt under lagring ved 4 °C i opptil 42 dager.

De ulike prosesseringsmetodene viste at HPP ved 600 MPa ga lave bakterietall mellom dag 14 og 28 ved lagring. På den andre siden resulterte 400 MPa til høye bakterietall allerede mellom dag 7 og 14. MW resulterte i høye bakterietall allerede på dag 0 på grunn av utilstrekkelig oppvarming. Autoklaverte prøver holdt seg under deteksjonsgrensen (<2.3 log cfu/g) frem til dag 42. Alle tellinger av både aerobe og anaerobe sporer holdt seg under, eller nær deteksjonsgrensen ved lagring. HPP ved både 400 og 600 MPa fikk minst endringer i den totale fargeforskjellen under lagring. Blomkålens tekstur skilte seg ikke fra kontrollen under lagring for både HPP- og MW prøver, mens de autoklaverte prøvene hadde en signifikant reduksjon i tekstur. Drypptapet for HPP var betydelig høyere enn kontrollen, mens MW resulterte i lavest drypptap. For alle prosesseringene var tørrstoff-innholdet stabilt under lagring, men med få forskjeller mellom dem. Ernæringskvaliteten til HPP blomkål ved 600 MPa for 2 minutter, holdt seg høy frem til dag 28. Det samme gjaldt for MW prøver på dag 0, hvor man kunne se en økning i næringsverdien, trolig på grunn av forbedret ekstrahering av næringsstoffer. Autoklaverte prøver på dag 0 resulterte i henholdsvis 30, 27 og 40% reduksjon i antioksidativ aktivitet, totalt fenolinnhold og askorbinsyre.

Samlet sett viser resultatene i denne studien at HPP og MW har potensiale for å bevare kvaliteten på blomkål sammenlignet med konvensjonell autoklavering. Optimalisering av prosesseringsbetingelser for MW-oppvarming er nødvendig. For begge behandlingene er det et potensiale for å legge til synergistiske behandlinger for å forlenge holdbarheten ytterligere, samtidig som kvaliteten kan bevares.

Preface

This thesis has been written as a final project for the 2-year Master of Science Degree in the field of Food science, technology, and sustainability (FTMAMAT) and comprises 45 ECTS credits.

The research project is the result of a collaboration between NTNU and Nofima and aims to investigate the potential of innovative processing technologies, high-pressure processing, and microwave with overpressure, compared to conventional thermal processing (retorting). Most of the experimental work has been conducted at Nofima, at the Department of processing technology in Stavanger, except for chemical analyses that were conducted at NTNU laboratory facilities.

Although the covid-19 situation has been challenging, the experimental work has been conducted as scheduled, with some of the following exceptions. Originally the high-pressure processing was supposed to be compared with several microwave conditions, but due to technical errors with the MW equipment, this was delayed and had to be revaluated. Delays of shipment resulted in some less analysis of the microwave treatment. Also, for the chemical analysis, technical issues with the freeze drier at Nofima led to fewer samples being analyzed.

It has been a pleasure to write this thesis at Nofima. Many thanks to my supervisor Marcin A. Kurek (NTNU), and co-supervisors Tone Mari Rode and Dagbjørn Skipnes (both from Nofima) for constructive help throughout the process. They have within their field of expertise provided me with valuable insight, guidance, and support. I would also like to thank the technical staff at Nofima, Laila, Karin, and Leena for their help and advice. Sincere thanks to Laila for helping me out with high-pressure processing and the retort process.

May 15th 2021, Trondheim

Christian Finnseth

“The science of today is the technology of tomorrow.”

Edward Teller, 1908 - 2003

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1. Introduction

Eating more vegetables is considered a good option for human health, but also the planet. According to the United Nations (UN) and Food and Agriculture Organization (FAO), we need to eat more vegetables to become more sustainable and reduce our climate footprint (FAO & Food Climate Research Network, 2016). According to the Norwegian Health Authorities, the national consumption of vegetables has increased over time, and in recent years from 2018 to 2019, the total consumption increased from 77.0 to 79.7 kg/person/year (Norwegian Directorate of Health, 2020). When it comes to the consumption of cauliflower in Norway, it was 1.81 kg pr. capita pr. year in 2018 (numbers not fixed for food loss) (Norwegian Fruit and Vegetable Marketing Board, 2019). Nevertheless, the Norwegian Government has set a goal to increase the consumption of vegetables by 20 % within 2021. This can contribute to better health and prevent diet-related diseases (Ministry of Health and Care Services, 2017).

Generally, standard cooking like heating is not recommended for vegetables because the nutrients are diluted and washed out with boiling water. Especially vitamin C is one of the most sensitive vitamins and is used to evaluate the influence of food processing on vitamin contents (Ahmed & Ali, 2013). Therefore, there is a constant need for the development of new kinds of technologies for the heating processes of vegetables. Innovative cooking treatments for vegetables can give the consumer safe, healthy, and nutrient food, and contribute to lower energy consumption (Pereira & Vicente, 2010).

Quality is a wide term; therefore, it needs to be concretized. According to Abbott (1999), «Quality implies the degree of excellence of a product or its suitability for a particular use». It is a human construct and encompasses sensory, nutritive, chemical, mechanical, functional properties and defects. One can also define quality from either a product orientation or a consumer orientation. In this assignment, the technical quality such as texture and color, drip loss, and the dry matter will be researched. Nutritional quality will also be checked, in terms of the content of ascorbic acid, total phenolic content, and antioxidant activity in cauliflower. Further, the microbial quality will also be investigated by assessing a shelf-life study for total aerobic counts and spores.

This master thesis will investigate how different use of high-pressure processing (HPP) and microwave treatment with overpressure (MW) affects the quality of cauliflower. For HPP, the combination of different times and pressures will be investigated and for microwave treatment, the independent variables will be the power of microwave and time of treatment. This will give some insight into what benefits can be achieved by using these new processing methods. The HPP and MW treated cauliflower will be compared with traditional processing, retort, to evaluate possible significant differences.

This work is a part of a project called iNOBox, whose goal is to investigate the potential of several innovative processing technologies and the effects on food quality. An industrial partner for this project wanted to explore whether the production of a

vegetarian ready-to-eat (RTE) dish with new technologies could be a solution for them. As a start, cauliflower was chosen to be the focus of this work. Today's solution gives a shelf-life of 35 days during refrigerated storage, and an increase for this was important for the partner.

1.1 Background

In the national action plan for a better diet (2017-2021) there is a goal to increase the consumption of vegetables by 20 % by 2021. Meanwhile, according to one of the recent surveys regarding the Norwegian diet, only about 14 % of adults eat the recommended number of vegetables. At the same time, 45 and 67 % of all men and women (respectively) above the age of 18 years eat the recommended intake of meat, where 25 % of men eat twice as much meat as advised (Ministry of Health and Care Services, 2017). During the last 10 years, the consumption of vegetables in Norway has increased, also from 2018-2019 (Norwegian Directorate of Health, 2020).

The four most important food values for the European consumer when choosing what to eat are food safety, price, taste, nutrition, and naturalness (Bazzani, Gustavsen, Nayga, & Rickertsen, 2018). New processing technologies can potentially have most of these values, but the price can be increased. Consumption of vegetables is associated with a lower incidence of chronic diseases such as cancer, and cardiovascular diseases. This has been shown through several studies (Manchali, Chidambara Murthy, & Patil, 2012; van Poppel, Verhoeven, Verhagen, & Goldbohm, 1999), and has also been shown to have clear nutritional health benefits (Kris-Etherton et al., 2002; Verkerk et al., 2009).

If new processing technologies, such as HPP and MW can contribute to better quality and prolonged shelf-life of processed vegetables, and thereby increased consumption of vegetables, this will be beneficial from both a health perspective and a sustainable point of view.

1.2 Objective

This research aims to investigate the potential for HPP and MW processing of cauliflower and how this technology affects the quality compared with conventional thermal processing. Different instrumental methods will be used to evaluate the quality. Microbial shelf-life, texture, color, dry matter, drip loss, and chemical content of selected nutrients will be examined.

Based on this, the following hypothesis were assumed:

(1) HPP is a technology that insignificantly changes the quality of vegetables in terms of physical and chemical changes compared to raw materials. (2) Microwave is not responsible for the loss of nutrients and color in the processing of cauliflower. (3) HPP and MW can increase the shelf-life of cauliflower compared with traditional heat processing.

1.3 Overview of experiments

A short overview of the different experiments conducted for the cauliflower is shown in Figure 1 below. Further details for experimental design can be found in the method section, section 3.2.

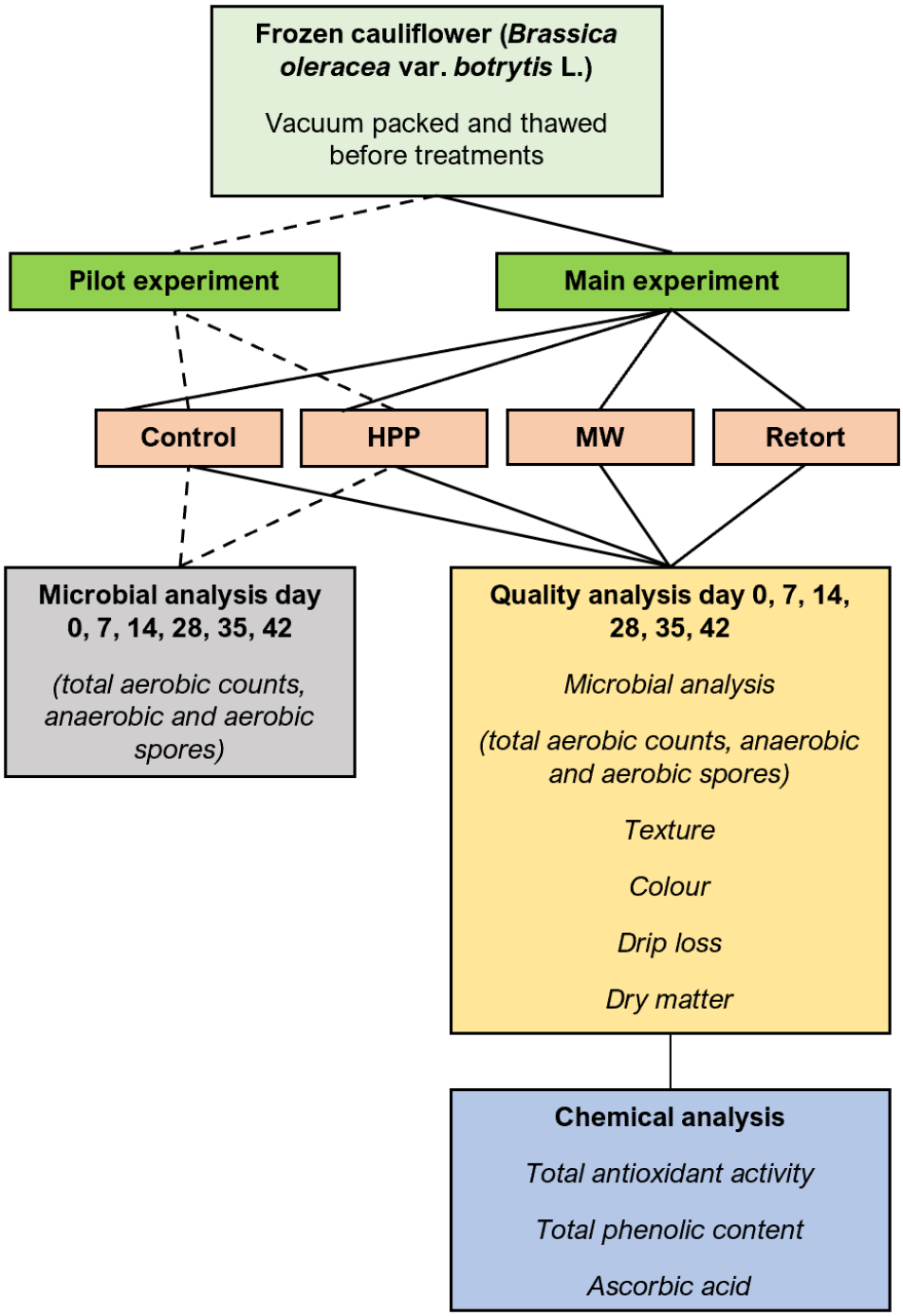

Figure 1. Short summary of the different experiments conducted on cauliflower.

2. Theory

2.1 Cauliflower

Cauliflower, also known as *Brassica oleracea* convar. *botrytis* var. *botrytis,* is a variety of cabbage within the *Brassicaceae* family. It consists of several flowerheads that are merged to the distinctive «head» of the cauliflower. The most common variant has a white color, but other variants with a purple or green color also appear (Great Norwegian Encyclopedia, 2020). According to the Norwegian Fruit and Vegetable Marketing Board (2019), the Norwegian consumption of cauliflower has been stable throughout the years, with 1.9 kg/capita/year in 2019, with an increase of 0.9 % from 2010 to 2019.

Cauliflower and other *Brassicaceae* vegetables are especially rich in bioactive compounds such as glucosinolates (GLSs), vitamin C, and polyphenols (Verkerk et al., 2009; Volden, Bengtsson, & Wicklund, 2009), and are known to present strong antioxidant properties (Podsędek, 2007). GLSs (Figure 2) is a class of phytochemicals claimed to be the active components for many of the physiological effects in different studies such as in vitro (within cell cultures), human, epidemiological, and animal studies, and could protect against cancer (Podsędek, Sosnowska, Redzynia, & Anders, 2006; van Poppel et al., 1999). The content of GLSs in cauliflower can vary greatly both within the same cultivation and between, due to several factors such as environmental factors (soil, climate, and fertilization), but the most important factor determining GLS content is genetic variation (Verkerk et al., 2009).

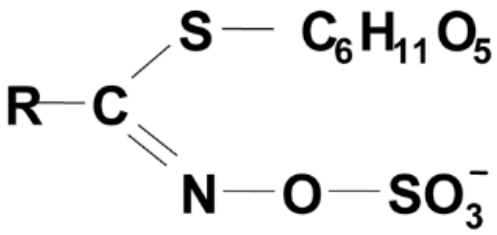

Figure 2. This shows the general structure of glucosinolates (GLSs). The side chain, R, defines whether the GLSs are classified as aliphatic (known as non-aromatic hydrocarbons), aryl, or indole. Retrieved from Verkerk et al. (2009).

2.2 Processing of plant-based foods

Plant-based foods are often, before consumption, subjected to cooking or processing to increase their edibility and palatability. Processing also aims to prolong the shelf-life of greens while preserving the nutritional and sensory profile of the food to the highest degree, without putting a constraint on the microbial safety of the food (Oey, Lille, Van Loey, & Hendrickx, 2008).

Today's conventional thermal methods for preserving food can often lead to a reduction in the sensory and nutritional profile. Therefore, in recent years, there has been a wider interest in looking into new processing methods such as high-pressure processing and microwave processing of foods, amongst others. The food industry is very active in technological innovation and over the last two decades novel non-thermal processing technologies have been viewed as useful for microbial inactivation while maintaining the quality of fresh and processed fruits and vegetables (Lafarga, Bobo, Viñas, Collazo, & Aguiló-Aguayo, 2018). The following sections give an overview of the different processing techniques that will be used for processing cauliflower and their effects on food quality.

2.3 Traditional thermal processing; retorting

The «father» of retorting is known as Nicholas Appert, who invented the use of heat to preserve food for the French military in 1810 (Featherstone, 2016). Today, the use of static retort is a widely accepted food processing technique, and over the last decades has provided the food industry with a predictable heat load that ensures food safety (Ates, Skipnes, Rode, & Lekang, 2014). According to Zhang, Tikekar, Ding, Gilbert, and Wimsatt (2020), traditional processing methods such as heating are still regarded as the most effective pathogen control for the food industry.

2.3.1 Principles

Retorting is a method of preserving food by heating it in hermetically sealed packages for a specific time at a specific temperature to achieve sufficient elimination of pathogens (Joseph, 2012). The process can often be referred to as canning, but other containers are also widely used, such as glass jars, foil laminated pouches, and plastic trays (Joseph, 2012). The ideal choice of packaging depends on the requirements of product type, processing conditions, required shelf-life, and target market (Featherstone, 2016). Today, there are many ways of retorting food, with advanced control options to manage the process. Combinations of steam, raining water, and rotation are possible, depending on the product. Modern retorts can achieve temperatures up to 145 °C, giving faster heating of the product (Awuah, Ramaswamy, & Economides, 2007).

When heating foods, it is a requirement that the coldest point in the product has achieved target lethality rates for inactivation of microbes. The duration of heating to sterilize or pasteurize food with a retort will depend on the following conditions (Fellows, 2009): (1) heat resistance of microbes and enzymes that are likely to be present in the food, including the numbers, concentration, and activity of these; (2) heating conditions; (3) the acidity of the food; (4) the thickness, shape, size of the packaging; (5) the thermophysical properties of the food (such as specific heat capacity). Further, the penetration of heat is also important and will depend on several factors, such as type of packaging (shape and size), type of food (high-fat content result in poor

conduction of heat), heating medium, filling medium (water/steam), the temperature gradient between packaging and the retort, ratio of products in the package, arrangement of the packages inside the retort, and the distribution of steam (Joseph, 2012). These factors will again influence the effectiveness of pasteurization or sterilization. Solid foods are mainly heated by conduction during retorting, while liquid and semi-liquid foods are mainly heated by convection (Fellows, 2009).

Industrial pasteurization or sterilization processes are often developed with the use of calculated F-values, to ensure that the cold spot in the food gets a sufficient reduction of a target bacterium (Tang, 2015). The formula below can be used to calculate the lethality of a given bacteria, where *F* is the accumulative time (min) exposure of food to a reference temperature (T_{ref}). For pasteurization, a typical reference temperature is often 70 or 90 °C (ECFF, 2006). *T (t)* indicates the temperature profile of the least heated location in the food (cold spot), and *z* (in °C) indicates how the thermal resistance of a target bacterium changes throughout the heating. Non-proteolytic *Clostridium botulinum* type E is a common bacterium used for retorted food that has a pH above 4.6, with a *z*-value of 10 °C (Peng et al., 2017).

$$\mathrm{F} = \int_0^t 10^{\frac{T(t)-T_{ref}}{z}}\, dt$$

2.3.2 Advantages

Compared to more novel food processing techniques, retort heating can process large volumes of food at once (Featherstone, 2016). Depending on the desired destruction of target pathogens, retorting with temperatures between 115 and 121 °C (called sterilization) could kill bacterial spores, enabling a shelf-life over several years in room temperature (Awuah et al., 2007). This can be viewed as economically efficient and can help save energy with cooling. In some cases, with the right processing conditions, such as high-temperature short time it could be possible to retain the nutritional value close to the fresh product, but this is easier to obtain in more liquid food (Featherstone, 2016; Rickman, Barrett, & Bruhn, 2007). Lipids, carbohydrates, and minerals are less affected by heating (Awuah et al., 2007). Retorting can also be applied to pasteurize RTE foods, providing a long shelf-life of several weeks when stored refrigerated. A long shelf-life is positive by minimizing food waste. Since several types of food are seasonal, retorting allows such products to be available for longer periods (Featherstone, 2016).

2.3.3 Limitations

Traditional static heating using retort has some limitations due to slow heat penetration in foods, especially for solid and viscous foods (Fellows, 2009). This can often result in significant overprocessing and longer processing times to ensure that a safe product is obtained. Other weaknesses are lack of consistency in texture and flavor, color, and

appearance of the processed food (Ates et al., 2014; Awuah et al., 2007; Bregje Krebbers et al., 2003). Too severe heat treatments, such as when achieving sterilization, can impair product quality. When heating foods at high temperatures, especially water-soluble vitamins (such as vitamin C and B) can be significantly reduced (Awuah et al., 2007). Another concern is that retorted vegetables can have a high sodium content because salt brine is often added to enhance the flavor and shelf-life (Featherstone, 2016).

2.4 High-pressure processing (HPP)

The first data on the effects of HPP on microbes were first described by Certes (1884). He found viable bacteria in water samples from a depth of 5100 m, with a pressure of approximately 50 MPa. Based on this observation, he continued studies on HPP equipment up to 600 MPa (Knorr, 1995). The use of HPP to inactivate bacteria in food was first used at the end of the 19th century, by the American Bert Hite (1899). He was the first to treat milk, vegetables, and fruits with very high pressure (Knorr, 1995) For instance, he extended the shelf-life of raw milk to 4 days after HPP at 600 MPa for 1 hour at room temperature (Farkas & Hoover, 2000).

However, it took several years to commercialize the HPP of foods, with Japan as the first country to use it in 1990 (Farkas & Hoover, 2000). Several studies have proven that HPP can be effective at an industrial level, and the technology has gained more interest in the latest years (Zhang et al., 2020). The type of foods that is on the market varies from baby food, smoothies, and juices to different RTE meals (Abera, 2019). The technology is mostly used in the North - American region, and it is approved by the FDA (U.S. Food and drug administration) (Wang, Huang, Hsu, & Yang, 2016). In Europe, the use of this technology has been slower, due to a more restrictive attitude towards novel food, where the regulation required several tests to prove the food to be safe. This has slowed down the commercialization of HPP in Europe (Tonello, 2011).

Using HPP can in some type of foods result in less use of preservatives, and it is therefore seen as a commercial advantage. It is expected that the use of HPP will increase further and will lead to several innovations in the food industry (Wang et al., 2016). HPP is batch processing, which puts limitations on the volume being put through daily. Therefore, the type of food should get an increase in value to defend its use.

2.4.1 Principles

HPP involves using liquids, often water, to build up pressure at 100 to 800 MPa, using pre-packed food in a flexible container (often in a vacuum), where pressure is built up by a hydraulic pump (see Figure 3). The pressure is transmitted inside the vessel to the food uniformly and at the same time, resulting in food products without overtreated parts. Typically, some temperature increase will occur in the vessel due to adiabatic pressure, typically 3 °C per 100 MPa (Tonello, 2011). Pressures under 100 MPa are

not much used, and for industrial purposes use pressures from 200 to 600 MPa, depending on the food (Fernandes, Casal, Pereira, Ramalhosa, & Saraiva, 2017).

The pressures are applied at refrigerated (cold pasteurization) to moderate temperatures below 60°C. Under refrigerated and ambient temperatures, HPP is considered a non-thermal process. If temperatures (≥40-50 °C) are used in combination with the high pressure, then it is known as pressure-assisted thermal processing (PATS) (Escobedo-Avellaneda et al., 2011).

Wang et al. (2016) mention several physical changes that happen with the food during HPP such as the formation or destruction of hydrogen bonds, electrovalent bonds, and hydrophobic bonds. Also, enzymes, starch, and proteins can be destructed, denatured, or gelatinized, and bacteria and other microbes can be destroyed in the food. This affects the edibility of foods and can give rise to different textures and lengthens the expiry date.

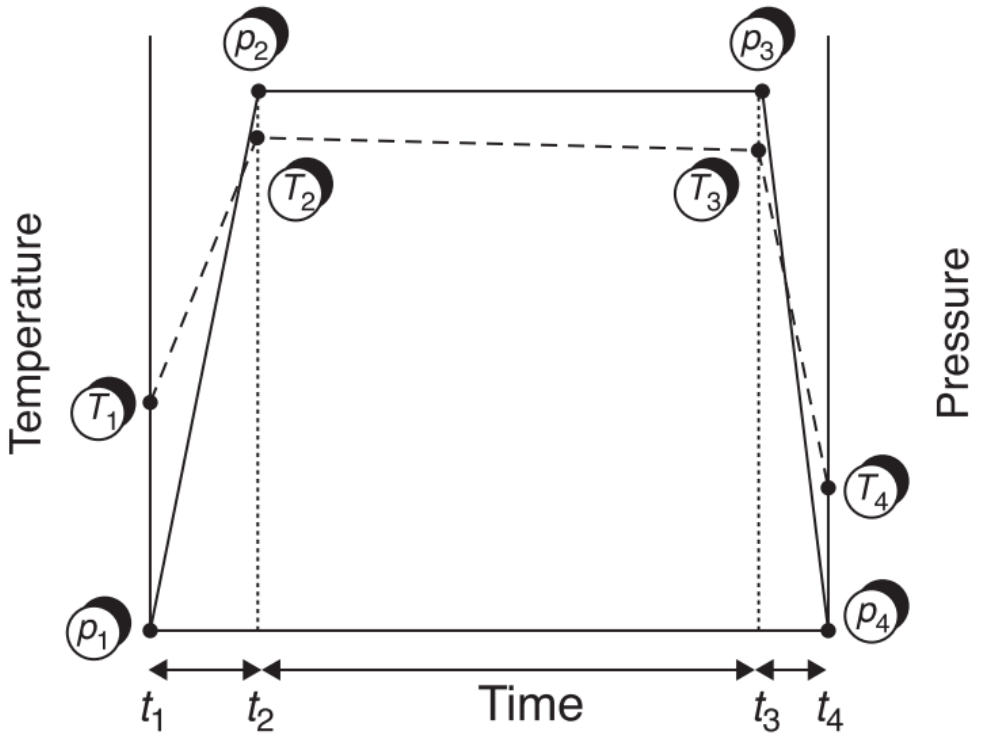

Figure 3. This is an overview of a typical pressure-temperature response of a water-based food matrix undergoing HPP. Come-up time, t1–t2; holding time, t2–t3; decompression, t3–t4. Retrieved from Tonello (2011).

2.4.2 Advantages

HPP offers processing at refrigerated and ambient temperatures. Depending on the pressure level used, HPP can prolong the shelf-life and effectively inactivate pathogenic microbes (microbes that can cause an infection or food poisoning) (Woldemariam & Emire, 2019). The distribution of pressure is uniform and independent of the size and shape of the product, and process times can be kept short (Tewari, Sehrawat, Nema, & Kaur, 2017). It is mainly used to inactivate microbes and enzymes,

with better preservation of flavor and nutrients (Escobedo-Avellaneda et al., 2011). It can also be applied to minimize losses of beneficial ingredients to give a distinct character to different foods (Santos et al., 2019). Further, the technology offers a lower effect on flavor, and color compounds, and texture of food products, compared to the same compounds caused by thermal pasteurization (Wang et al., 2016). HPP also offers a lower energy use compared to traditional preservation methods such as the use of heat (Rode & Hovda, 2012). Moreover, HPP food can provide the consumer with a «clean label» product, free from preservatives, and be marked as healthy and natural (Hovda, Vangdal, & Rode, 2014).

2.4.3 Limitations

Using HPP of foods for batch processing puts limitations on the volume being put through daily. Therefore, the type of food should get an increase in value to defend its use. There are also high investment costs for HPP equipment, but costs associated with the daily use are considered fairly low (Rode & Nilsen, 2014). Foods with very low moisture content will have inefficient inactivation of microbes using HPP (Heinz & Buckow, 2009). In addition, foods with a high pH (low acid) need to be stored refrigerated to slow down the growth of spores (Black et al., 2007). This can be avoided by adding different preservatives, reducing pH, or adding a heating step. Nevertheless, most HPP food on the commercial market today is kept refrigerated (Abera, 2019). Products containing air cavities, such as strawberries can be a problem to process, due to collapsing (Hovda et al., 2014). Further, HPP requires specific airtight and flexible packaging that limits the use to an extent (Fernandes et al., 2017). As such, glass and some types of metal packages cannot be used.

2.5 Microwave heating (MW)

2.5.1 Principles

The limitations of retorting solid foods have been a key driver for the development of microwave heating for preserving food (Tang, 2015). Microwave is a type of electromagnetic wave of frequencies between the range of 300 MHz and 300 GHz (Regier, Knoerzer, & Schubert, 2017). MW heating commonly operates at frequencies of 915 or 2450 MHz. In Europe, only frequencies at 2450 MHz are allowed. It has been shown that 915 MHz can give deeper penetration depth of several types of foods compared to 2450 MHz, but 2450 MHz will be used focused on from now on (Vadivambal & Jayas, 2010).

The mechanism behind how microwaves behave in different kinds of foods is complex and is still not fully understood. Swamy and Muthukumarappan (2021) point out that food heated by microwaves is affected by the initial temperature of the food. They further claim that dielectric properties in food change with temperature, depending on the type of food matrix, and its physical properties. As an example, Tang (2015) mentions that dielectric properties dramatically change when comparing the MW heating of

frozen and thawed food. This results in more MW power is reflected from moist than frozen foods. The author explains this with that frozen water molecules are trapped in a rigid structure, causing a lower dielectric loss factor (this factor decides the conversion of MW energy to thermal energy). Further, the temperature and moisture content in food can change during heating and can have combined effects on each other (Tang, 2015). Nonhomogeneous products are subjected to uneven heating, and when the thickness of the product is close to the wavelength, a higher temperature can be achieved (Swamy & Muthukumarappan, 2021).

According to Rosnes, Skåra, and Skipnes (2011) the MW heating effect in high-moisture food is based on polarization losses due to water reorientation and to electric conductivity of electrolytes formed by ions in the water fraction of the food. Tang, Hong, Inanoglu, and Liu (2018) explains this in detail: since water is a polar molecule, it will have electric dipole moments when subjected to microwaves. The positive and negative charges will try to align with the changes in the electromagnetic field, and a rotation of the polar molecule occurs. This is known as dipole rotation, and the friction among molecules converts electromagnetic energy into heat, causing a rise in the temperature of the food. Ions (e.g., salts) present in the food will also generate friction and heat due to ionic conduction. Instead of rotation, ions present in the food get a forward and backward motion that results in friction (Swamy & Muthukumarappan, 2021).

2.5.2 Advantages

Applying MWs to foods has the advantage of better retain the color, taste, quality, and nutritional value compared to food cooked with conventional methods (Swamy & Muthukumarappan, 2021). Compared to conventional heating methods, MW heating can also give faster heating rates, reduced processing times, and more efficient water removal of foods (Tang et al., 2018). It also offers a lower energy consumption compared to conventional heating, because only the food itself is heated during MW while retorting needs to heat up and cool down water, steam, and equipment. In some cases, MW also has proven to be effective in the destruction of pathogens and by inactivation of enzymes (Contreras, Benlloch-Tinoco, Rodrigo, & Martínez-Navarrete, 2017).

2.5.3 Limitations

There is still a need for a deeper understanding of how MWs heat food. Lack of uniformity of heating is still a problem, though several techniques such as rotation of the food, and cycling of the power can improve the heating (Swamy & Muthukumarappan, 2021). For some types of foods, overheating can occur, resulting in a quality loss (Liburdi, Benucci, & Esti, 2019). In others, uneven heating can lead to cold spots where potential pathogens can survive and reduce the quality (Cho & Chung, 2020). Type of food packaging can also be a limiting factor, where they need to be transparent to electric fields (primarily made from plastic films). MWs also have limited penetration depth (a few millimeters), resulting in a larger volume of food that cannot be heated at once (thus minimizing the capacity of food producers) (Rosnes et al., 2011).

2.6 Effect of processing on quality

There exist several ways to measure the quality of vegetables, where often flavor, color, texture, and nutrition are some of the most important parameters to assess (Fernandes et al., 2017).

2.6.1 Retort

As earlier mentioned, traditional heating of food can lead to several negative effects on the quality. When looking at the color, high heat over a long time will be able to degrade the different pigments of processed vegetables (Awuah et al., 2007). For instance, Coultate (2009) points out that chlorophyll in green plants can lose its fresh-like appearance and turn brown. He explains that because heating in the presence of acids causes loss of Mg^{2+} ion in the chlorophyll, resulting in pheophytins a and b that have a dirty brown color. Natural occurring acidity in plant cell vacuoles makes this phenomenon difficult to avoid during heating. By adding small amounts of sodium carbonate, it is possible to remain color, but this has negative effects on the texture, flavor, and content of ascorbic acid (Coultate, 2009). Awuah et al. (2007) mention that Maillard reactions also can cause color changes during heating, with complex reactions between proteins and reducing sugars. This could also lead to loss of amino acids, thus reducing the nutrients present in the food. High losses of nutritional quality during retorting of vegetables have been extensively reported by Rickman et al. (2007).

For the texture of foods, retorting often results in an overcooked appearance, with consequent loss of their juicy and crispy character. Plants get their distinctive texture depending on the product, but these factors are general for most plants (Terefe, Buckow, & Versteeg, 2013): (1) pectin acts as a cement for the cell walls, and gives firmness and elasticity; (2) cellulose-hemicellulose network makes the basic structure of the cell wall and gives structure and shape; (3) different compounds within and between plant cells affects the texture (e.g. vacuoles, air gaps, and presence of starch); (4) turgor pressure within cells are generated by osmosis and gives crispiness. When heating low-acid plants above 80 °C depolymerization of pectin (due to beta-elimination degradation) becomes significant, leading to softening of the texture (Sila et al., 2009). This is the main cause of texture loss, but a loss of turgor pressure will also influence (Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009).

2.6.2 HPP

For plants, HPP can disturb cell permeability, resulting in the movement of water and other metabolites in the cells. How much the cell is disrupted is dependent on the pressure being used, but also the type of plant cell (Oey et al., 2008). Small molecules, which have no secondary, tertiary, and quaternary structures, such as amino acids, vitamins, and flavor and aroma compounds remain unaffected by HPP (Xin, Zhang, Xu, Adhikari, & Sun, 2015) When it comes to HPP of cauliflower, the literature is quite scarce and incomplete. Due to this, effects on other vegetables within the *Brassicaceae* can be investigated.

Textural changes in vegetables during HPP are related to changes in the cell wall (enzymatic and non-enzymatic reactions) and the pressure itself. This is dependent on the structure of the cell wall and the space between the different cells (Norton & Sun, 2008) When applying pressure on plant tissue, the cell disruption can lead to different high molecular compounds leaking and interacting from different plant cells (Terefe et al., 2013). This can lead to softening and is also caused by loss of turgor pressure (loss of gas-filled in cells) (Oey et al., 2008). Nevertheless, compared to thermal processing, HPP results in minor changes in pectin depolymerization (Van Buggenhout et al., 2009).

Biochemical changes also play a role in the change of texture, color, flavor, and nutritional value during food processing, including HPP. Terefe et al. (2013) give an overview of important plant-related enzymes, were: (1) Polyphenol oxidase (PPO), peroxidase (POD), and lipoxygenase (LOX) can induce change in color and flavor; (2) pectinase, cellulase, and hemicellulose can change the texture; (3) PPO, POD, and ascorbic acid oxidase can affect the nutritional quality. Among the class of pectinase, pectin methyl esterase (PME) could lead to softening during HPP, but in some cases, it could also enhance the texture of vegetables (Terefe et al., 2013). Pressures above 400 to 600 MPa combined with heat (50 °C) has been shown to inactivate the enzyme (Norton & Sun, 2008). Temperature, pH, solids, and the presence of other proteins can also affect the stability of enzymes during HPP (Norton & Sun, 2008).

De Roeck et al. (2009) found that high temperature in combination with high pressure (500, 600, and 700 MPa/90, 110, and 115 °C) could result in better retention of the texture of vegetables. This was because their experiment gave less beta-elimination of the pectin molecule and that the process stimulated demethoxylation of pectin. Since low methoxylated pectin can strengthen the plant tissue (by forming cross-links with calcium ions), preservation of texture was found.

In general, HPP preserves the color of many types of foods, but some pressure-resistant enzymes and bacteria can lead to degradation of the color during storage and result in browning (Krebbers, Matser, Koets, & Van den Berg, 2002). This is especially the case when using very high pressures (Fernandes et al., 2017; Oey et al., 2008). On the contrary, it has also been shown that pressure applied at low and moderate temperatures can preserve pigments such as carotenoids and chlorophyll (Castro, Saraiva, Domingues, & Delgadillo, 2011).

2.6.3 MW

Processing of plants with the use of MW has in several cases been shown to enhance the quality. As for HPP, few studies have been conducted on MW of cauliflower, but there exists a few. In general, compared to traditional heating, MW heating of vegetables has been found to preserve color and retaining high levels of nutrients such as bioactive components and antioxidants (Guo, Sun, Cheng, & Han, 2017). Nevertheless, the method used for heating will affect the nutritional quality. As an example, when MW

vegetables in water, greater losses have been reported (Barrett & Lloyd, 2012). In contrast, when heating broccoli without water, Severini, Giuliani, De Filippis, Derossi, and De Pilli (2016) found high retention of total phenolics and ascorbic acid.

Since MW in many cases can heat foods more rapidly than retorting, this offers advantages for preserving texture and color (Stanley & Petersen, 2017). The heating will depend on several factors as described in section 2.5. Consequently, the quality will be a function of raw materials, type of process, and processing conditions. The preservation of texture and color during MW of vegetables have been widely reported by several authors (Brewer & Begum, 2003; Latorre, Bonelli, Rojas, & Gerschenson, 2012; Liburdi et al., 2019; Zhong, Dolan, & Almenar, 2015; Zhong et al., 2017).

2.7 Food safety & shelf-life

In general, most vegetables present ideal environments for the survival and growth of microbes. They have mostly a pH near neutrality (approx. pH 5.0 to 6.8) (Featherstone, 2016), and the internal tissue is nutrient-rich (Barth, Hankinson, Zhuang, & Breidt, 2009). Most microorganisms present on vegetables are from the soil, air, or through irrigation water. Spoilage microbes and pathogens can be introduced in several ways: through the seed, during growth, harvesting, post-harvest, or during storage, distribution, and further handling (Barth et al., 2009). Spoilage microbes affect the food by changing the flavor, smell, appearance, and texture of vegetables, therefore it is important to control the growth of these. By using measures such as good agricultural practices, good manufacturing practice, and good hygiene practice throughout the value chain of vegetables, food safety and shelf-life can be strengthened (Barth et al., 2009).

Fresh cauliflower, such as others within the *Brassicae*, has a short shelf-life after harvest due to its high respiration rate and perishability (Giuffrida, Agnello, Mauro, Ferrante, & Leonardi, 2018; Lucera, Costa, Mastromatteo, Conte, & Del Nobile, 2011; Sousa-Gallagher, Tank, & Sousa, 2016; Vaishnav, Adiani, & Variyar, 2015). The main quality losses are tissue browning, floret opening, firmness loss of stem, and the development of undesirable odors (Zhan, Hu, Pang, Li, & Shao, 2014). These are physiological responses of the plant tissue, but microbes will also contribute to a decrease in quality and shelf-life. Because of this, further preservation of cauliflower is needed, often in terms of refrigeration, packaging, or freezing (Vaishnav et al., 2015).

Before the food is put out on the market for consumption, it needs to be proved to be safe to eat. According to the European Chilled Foods Federation (ECFF, 2006), the shelf-life for the RTE meal must cover the manufacture, distribution, warehousing, display, and consumer use while remaining safe to eat and remain high quality. It is therefore important to assess shelf-life when new technologies are investigated (Stanley & Petersen, 2017).

Total aerobic bacteria count (TAB) can be used as an indication for the spoilage of foods (Fung, 2009). According to EC 2073/2005 (2008), chapter 2.1.6, there are limits for the lower and upper growth of aerobic total counts, 5.69 (log cfu/g) and 6.69 (log cfu/g), respectively. These limits are for ground beef but can be used as an indication for when other food products are not safe for consumption. The values are not satisfactory if one or more samples tested are above 6.69 (log cfu/g). These numbers are also following others, were Fung (2009) claims that for solid vegetables TAB of 0-2 log cfu/g (low count), 3-4 log cfu/g (intermediate count), 5-6 log cfu/g (high count), and 7 log cfu/g (spoilage).

For products that are to be heat-treated, EC 2073/2005 (2008) states that: «Any heat treatment process for processing an unprocessed product or for further processing of a processed product shall: a) heat all parts of the product being treated to a given temperature for a given period of time (…) ». This is essential for providing safe food, where the standardization of time and temperature are critical control points.

Regarding the shelf-life of pasteurized RTE foods in general and vegetables, there does not appear to be a universally accepted standard for all products (Peng et al., 2017). Shelf-life is defined as «the period of time for which a product remains safe and meets its quality specifications under expected storage and use conditions» (ECFF, 2006). There are numerous risks associated that need to be considered. For instance, non-proteolytic *C. botulinum* type E can grow at low temperatures (3.3–10 °C) and in a low oxygen environment and is, therefore, a risk considered (Hong, Huang, Yoon, Liu, & Tang, 2016). *Listeria monocytogenes* can also be a target bacterium (Tang et al., 2018).

2.7.1 HPP

Because the cell membrane of vegetative bacteria is damaged during HPP, preservation is obtained (Norton & Sun, 2008). Refrigeration of HPP food is common, therefore especially psychotropic microbes are of concern both for spoilage and possible foodborne illness (McClements, Patterson, & Linton, 2001).

Pressures up to 600 MPa has been shown to inactivate vegetative pathogens and spoilage bacteria and yeast and molds (Woldemariam & Emire, 2019). On the other hand, spores can survive pressure treatments up to above 1000 MPa, and some enzymes are also pressure-resistant (Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015; Chakraborty, Kaushik, Rao, & Mishra, 2014). It is generally recognized that high pressure gives an increased food safety, but it is dependent on the food being investigated (Serment-Moreno, Barbosa-Cánovas, Torres, & Welti-Chanes, 2014). The food must contain certain amounts of water to achieve an effective reduction of microbial counts. Bacterial inactivation will also depend on the type of microbe, food composition, pH, and water activity of the food (Serment-Moreno et al., 2014). Variations in pressure resistance can also be seen among different strains of bacteria. Cells in a stationary growth phase are more resistant than those in the exponential growth phase

(McClements et al., 2001). Also, Gram-positive bacteria are more resistant to pressure than Gram-negative bacteria (Tonello, 2011).

Pinton, Bardsley, Marik, Boyer, and Strawn (2020) found that raw cauliflower has been associated with recalls due to the potential of *L. monocytogenes* contamination. This bacterium is ubiquitous in soil and therefore a potential risk, but several processing steps can reduce the presence of this microbe. Arroyo, Sanz, and Préstamo (1997) found that HPP cauliflower (300/350/400 MPa, 5 °C, 30 min) gave no growth of TAB. They also subjected a pure culture of *L. monocytogenes* for 350 and 400 MPa, this decreased the population below the detection limit. HPP is a technology that can eliminate or inactivate vegetative microbes, but it needs to be tested out on each specific product to evaluate the safety (Fernandes et al., 2017).

2.7.1.2 Effects on spores

When it comes to spores, it is well established that they can be very pressure resistant (Norton & Sun, 2008). The process of initiating a spore condition is highly complex and coordinated. It enables endospore bacteria to survive in extreme conditions when access to nutrients and moisture is limited (Reineke, Mathys, Heinz, & Knorr, 2013). The multilayer morphology (Figure 4) of spores makes them withstand heat, radiation, drought, extreme pH gradients, and toxic chemicals (Setlow, 2003). Among spores, one of the most heat and pressure-resistant pathogenic bacteria is *C. botulinum*, but *Bacillus cereus* also has a high resistance to pressures and is also of concern (Black et al., 2007).

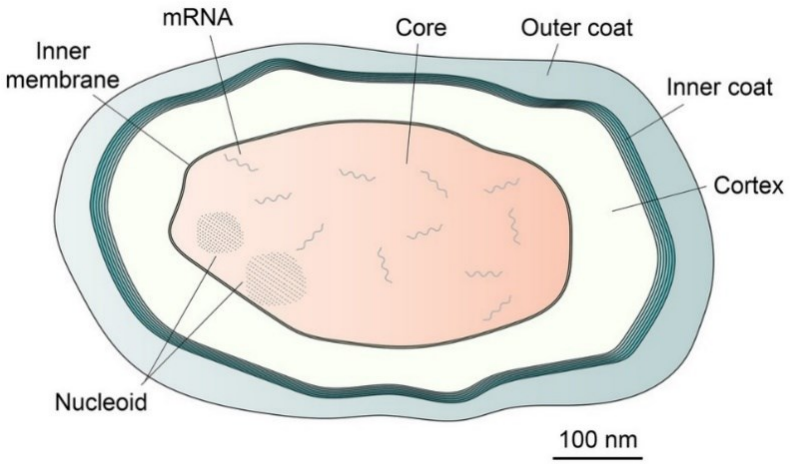

Figure 4. Spores share common morphological characteristics, as shown in this illustration. The coat can be divided into an outer and inner coat for some species. Retrieved from Setlow and Christie (2020).

Since pressure alone at ambient temperature has little effect on spore destruction, further processing steps are often needed (Farkas & Hoover, 2000). At the same time, pressure can trigger spore germination by several complex activation mechanisms (Black et al., 2007). In general, pressures from 100-400 MPa at 20–50 °C can trigger spore germination, but this varies and can be even higher for specific types of spores (Reineke et al., 2013). According to Borch-Pedersen et al. (2017), pressurization below 300 MPa triggers spore germination by activating germination receptors (GRs). They

further claim that pressures above 300 MPa likely trigger spore germination by opening dipicolinic acid (DPA) channels present in the inner membrane of the spores.

Once the spores germinate, they are often more vulnerable to heat treatments. Because of this, a combination of pressures and heat can inactivate the spore, known as a two-step inactivation mechanism (Balasubramaniam et al., 2015). Others have described an additional third step, called «the unknown state», which claimed to be a step between germination and inactivation (Borch-Pedersen et al., 2017).

Pressure in the range of 400-600 MPa in combination with a temperature above 70 °C has shown a synergistic effect on the inactivation of spores (Ates, Skipnes, Rode, & Lekang, 2016; Gao, Qiu, Wu, & Fu, 2011). By increasing the temperature up to 90-120 °C with 400-600 MPa, potential sterilization can be achieved, though more research is needed here (Balasubramaniam et al., 2015). Reineke et al. (2013) highlight the need to better understand how different bacterial spores react to temperature and pressure combinations. Still, a case-by-case approach is needed to maintain food safety.

2.7.2 MW

Microwave heating can be used in several ways to affect the shelf-life of foods. One common way is using microwaves for the pasteurization of foods. The definition of microwave pasteurization is «a process that rapidly microwaves heat-treats and extends the shelf-life of foods while minimizing quality damage compared to conventional externally applied heat sources» (Stanley & Petersen, 2017). In general, Rosnes et al. (2011) mentions that these considerations should be taken into account for pasteurized products using microwaves: (1) The time required for surviving microorganisms to germinate and reach undesired levels or produce toxins; (2) the time for enzyme activity or other chemical reactions to degrade the product to an unacceptable level; (3) for mild heat treatment, the processing might even accelerate the activity of some enzymes; (4) physical factors such as discoloring by light, among others.

According to Pendrous (2011), microwave in-pack pasteurization can give a shelf-life of up to 30 days. As for this experiment, the food will not be contaminated after processing, but remaining the cold chain intact is important (under 4 °C). To get a longer shelf-life, Stanley and Petersen (2017) claim that more intense processing is required. This can though lead to a less fresh-like appearance for the food.

Process temperature for 65-90 °C has been used for pasteurization, to get a 6 log inactivation of bacteria (Bozkurt-Cekmer & Davidson, 2017). A temperature of 72 °C for 2 min is used to achieve a 6 log bacterial inactivation for chilled products (Stanley & Petersen, 2017). When using vegetables that are non-uniform it is essential to find out the optimal heat treatment that gives heat in the coldest region. The core temperature of heat-treated products is usually the most critical point and needs to be measured by the insertion of thermocouples. When the coldest region has reached the target

temperature it needs to be held for a given period to inactivate the target bacteria (Bozkurt-Cekmer & Davidson, 2017), as described with the F-value in section 2.3.1.

2.8 Quality analysis of foods

2.8.1 Color

For many types of food products, the visual senses are the first to be used by the consumer (Kilcast, 2013). If a negative impression of the product is communicated, the consumer might reject the product. Color is considered the most important attribute of any food appearance (Pathare, Opara, & Al-Said, 2013). The observation of color will depend on the observer and the surrounding conditions, where a light source is needed to observe the color. For vegetables, the color can indicate the stage of ripeness. It can also be used as an indicator and predictor of other quality parameters, such as texture, heat load, and shelf-life, among others (Kilcast, 2013).

To maintain the quality of food, the color must be measured and standardized. Several instrumental analyses have been made for color measurements. By using color coordinate systems, it is possible to describe the color of food. One of the most common systems generates quantitative data based on the CIE (Commission Internationale d'Eclairage) L*a*b* system, known to provide a uniform color difference concerning the human perception of color (Kilcast, 2013). According to Granato and Masson (2010), the value of a* takes positive values for red colors and negative values for green tones. Further, the value b* takes positive values for yellow colors and negative values for the blue tones. Lastly, L* is an approximate measurement of the lightness of a sample, where the value goes from darkness (0) to complete lightness (100), with different tones of grey in between the scale.

When evaluating colors, it is important to determine how much one color differs from a reference, such as a control. To do this, Delta E (ΔE), also known as total color difference (TCD), can be calculated (see equation in section 3.4.2). Figure 5 illustrates the difference between two colors as two points in the L*a*b* spectrum. Differences in perceivable color by the human eye can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3), and small difference (TCD < 1.5) (Pathare et al., 2013).

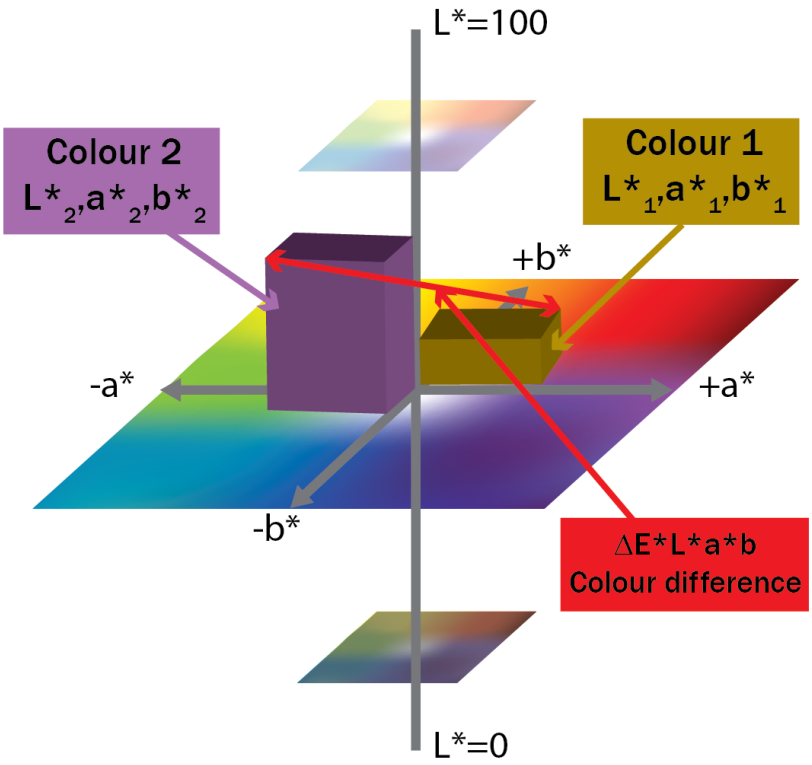

Figure 5. Illustration of the Lab color chart, with color differences shown. Retrieved from Martin (2015).

2.8.2 Texture

Providing RTE food that maintains the firmness of processed vegetables is important for the food producer, but also concerning consumer perception of the food. This can lead to a higher degree of likeability and appetite for the consumer (Granato & Masson, 2010).

There exist vast amounts of definitions of texture, but one of the hereby most accepted is by Szczesniak (2002): «Texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetic». Moreover, this definition states that: (1) Texture is a sensory property, and because of this, only humans can perceive and describe it. (2) It is a multi-parameter attribute (variety of characteristics). (3) It derives from the structure of foods, such as micro- and macroscopic or molecular basis. (4) It is detected by several senses that humans have, most importantly the senses of touch and pressure.

There are many ways of measuring the texture of vegetables, depending on what textural attributes one wants to address. This is because humans will perceive texture in several ways, such as using the response from muscles, teeth, and sounds (Chen & Rosenthal, 2015). The different textural methods cover several mechanical properties of the food such as chewiness, hardness, crispness, cohesiveness, and viscosity (Fillion & Kilcast, 2002). Some methods give a single output of data, such as the force needed to compress a sample, as seen in Figure 6. Other methods display several outputs of

data that can be correlated to the human sensory perception of texture. Proper selection of parameters to measure is essential to provide consistent and repeatable results (Kilcast, 2013).

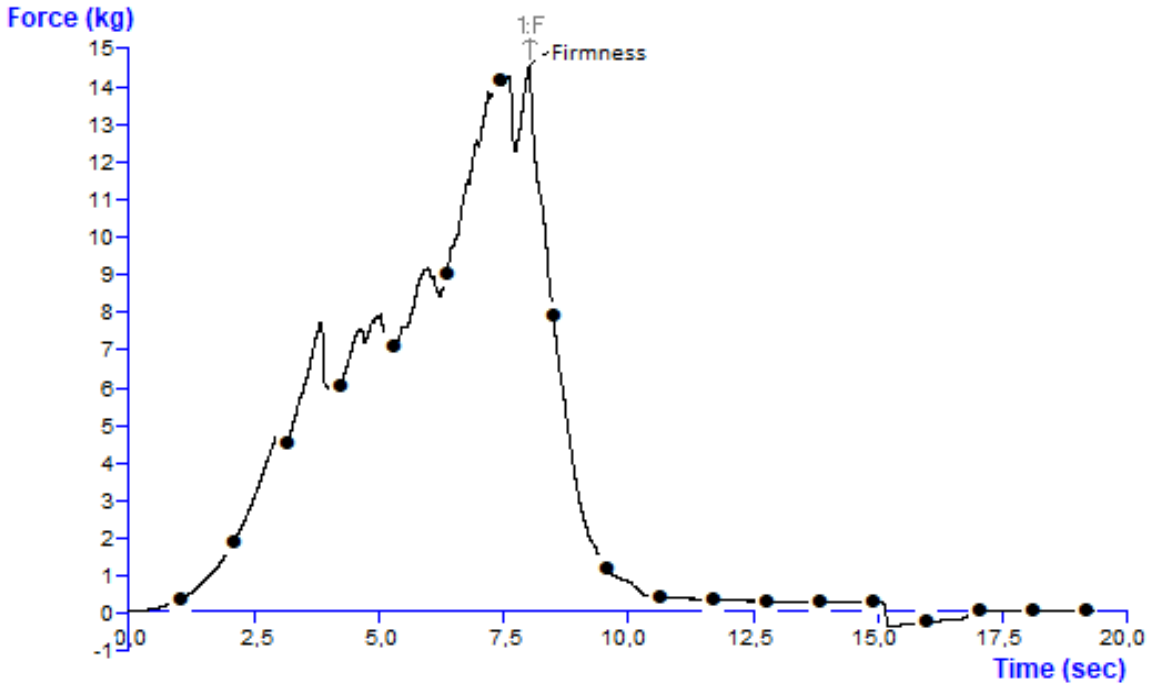

Figure 6. An example of a compression (kg force) of a sample over time (s), the highest peak force can mimic the first bite and is used to indicate the firmness of a sample. Under the curve shows adhesiveness, can indicate the viscosity of a sample. Retrieved from the TA.XTplus software.

2.8.3 Drip loss

High drip loss is not beneficial for food producers in several ways as it has a negative effect on quality (Wang et al., 2017): (1) it can lead to less weight of the product, and consequently lower price/kg; (2) it can result in poor texture and color; and (3) it has been correlated with loss of nutritional quality because it can indicate loss of water-soluble vitamins (Barth et al., 2009).

Drip loss is often closely related to the freezing, thawing, and heating of vegetables. Xin et al. (2015) explain that freezing results in the formation of ice crystals, which again lowers the turgor pressure of the plant cell. This leads to weaknesses of the cell wall, cell separation, and a soft texture, and high drip loss during thawing can occur. For further processing, such as heating, drip loss is also related to texture changes, as described in section 2.6.1.

Measurement of drip loss can be done in several ways. An easy way of doing this is by weighing the food before and after processing, to calculate the percentage difference in drip loss. The equation for this method can be found in section 3.4.4.

2.8.4 Dry matter

The dry matter (also known as dry weight) of foods consists of all the remaining compounds after removal of the moisture, until a constant weight is achieved (Wang et al., 2017). For some foods, the moisture content is used to indicate the quality. In plants, dry matter can consist of fats, proteins, carbohydrates, vitamins, minerals, and different antioxidants. There are several ways of drying foods to obtain dry matter, such as the use of vacuum ovens or convection ovens. The formula for calculating dry matter percentage can be found in section 3.4.5.

Content of dry matter often changes during processing and storage of vegetables (Rickman et al., 2007). According to Vervoort et al. (2012), a reduction in dry matter in vegetables can be a result of leakage of water-soluble substances, such as sugars, and degraded pectin. On the other hand, they claim that it can be a result of the absorption of liquids in between or into cells that have been denatured.

2.9 Nutritional analysis

It is well known that naturally occurring antioxidants can be significantly reduced by processing and storage. Especially thermal heat treatments are believed to be the main source of depletion (Číž et al., 2010).

Free radicals and other reactive substances (reactive oxygen and nitrogen species) are formed in the body continuously, especially through oxidative metabolic reactions (Nordic Council of Ministers, 2012). These reactive substances can develop because of diseases, or other external factors such as smoking, radiation, and pollution amongst others. Antioxidants are different classes of substances that can delay or prevent oxidation of substrates such as lipids, proteins, carbohydrates, and DNA (Dragsted et al., 2004). Valko, Rhodes, Moncol, Izakovic, and Mazur (2006) states that if these reactions are not prevented sufficiently by antioxidants, the free radicals can react and possibly change the structure and function of cell membranes, DNA, RNA, lipids, proteins, and carbohydrates. When the balance between endogenous antioxidant defenses and radicals is disrupted, the authors say oxidative stress occurs in the body. They further explain that prolonged oxidative stress has been associated with several diseases such as cancer, and cardiovascular diseases. In addition to the antioxidant defense system that takes place in the body (endogenous), intake of antioxidants through the diet might also contribute to the defense system (Nordic Council of Ministers, 2012).

Although there is a high degree of scientific evidence that a diet rich in fruits and vegetables reduces the risk of several chronic diseases associated with oxidative stress, there are no recommendations for eating specific antioxidant-rich vegetables and fruit per se (Nordic Council of Ministers, 2012). One of the reasons being the difficulties to reliably determine oxidative stress in vivo (meaning in living organisms) (Dragsted et al., 2004). Further, the antioxidant effects of different vegetables are highly variable and depend on the assay being used (Prior, Wu, & Schaich, 2005).

2.9.1 Analytical methods to measure antioxidants.

There are numerous methods one can apply to detect and measure the nutritional status of vegetables. Choice of method will depend on the knowledge, the available equipment, and cost and time available. The two first methods below are relatively easy and cheap analysis to apply, whereas the last one, HPLC, requires more competency of the equipment. An advantage is that the HPLC method is both accurate and precise (Volden, 2008).

Each of the below-mentioned methods aims to detect the antioxidant content. Briefly, Prior et al. (2005) mentions four general sources of antioxidants. The first is within enzymes, the second is within large molecules (different proteins), the third within small molecules (ascorbic acid, (poly)phenols, uric acid, tocopherol, carotenoids), and the fourth group is within some type of hormones. On the other hand, there are several free radical and oxidant sources, such as singlet oxygen or superoxide. Further, both oxidants and antioxidants have different physical and chemical features, that can react in several mechanisms. According to the authors, this poses some challenges when detecting antioxidants, because different antioxidants can react in a different way to different radicals or oxidant sources. Because of this, no single analysis will accurately reflect all antioxidants in a complex matrix such as different greens (Prior et al., 2005).

2.9.2 Antioxidant capacity (DPPH)

The 2.2-Diphenyl-1-picrylhydrazyl (DPPH) radical (see Figure 7) is one of a few stable organic nitrogen radicals, that holds a deep purple color (Olszowy & Dawidowicz, 2018). This type of assay is based on the reducing capacity of the DPPH$^{\bullet}$ radical reduction by the antioxidant and was first described by Brand-Williams, Cuvelier, and Berset (1995). During the DPPH$^{\bullet}$ inhibition, there is a loss of color causing a decrease in absorbance (Wang et al., 2017). The DPPH$^{\bullet}$ holds its violet color in ethanol or methanol, with maximum absorbance at 515 nm (da Silva Oliveira, Neves, & Ballus, 2019).

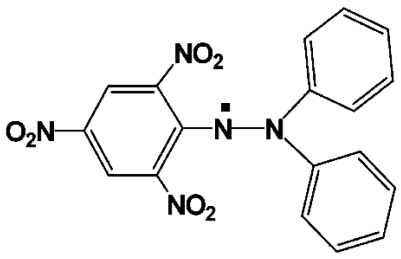

Figure 7. Chemical structure of 2.2-Diphenyl-1-picrylhydrazyl (DPPH). Retrieved from Prior et al. (2005).

The DPPH method is based on single electron transfer (SET) reactions, and they are known to be slow and require a longer time to reach completion (Prior et al., 2005). The SET reactions are very sensitive to ascorbic acid and uric acid, which can interfere/disturb the results. Also, trace components and different contaminants, such as metals, can interfere with the DPPH, and thus might result in variability, less reproducibility, and consistency of the results obtained (Prior et al., 2005).

Prior et al. (2005) mentions several benefits and limitations of the DPPH method. The advantage is that it is simple, rapid, and one only needs a UV-spectrophotometer to perform it. Some limitations are that the DPPH reacts as both a radical and oxidant, and the color can be lost due to either radical reactions or reduction (SET), but also regarding different unrelated reactions. Another major determinant for the reaction is steric accessibility, where small molecules (such as ascorbic acid), have better access to the radical site resulting in a higher antioxidant capacity with this test. Other issues being that DPPH can also be decolorized by reducing agents and hydrogen transfer, which can lead to inaccurate results from the analysis. Thus, several limitations need to be taken into consideration when interpreting the data from the DPPH assay.

2.9.3 Total phenolic content (Folin-Ciocalteu assay)

The composition of phenolics in vegetables varies greatly, and since they act as antioxidants they can be subjected to oxidation during storage and processing (Rickman et al., 2007).

To measure the total phenolic content of a sample, the Folin-Ciocalteu is a well-known way of doing this. The basic mechanism behind this is a reduction reaction of Mo^{6+} to Mo^{5+} (molybdenum oxides), which holds a blue color, and it can therefore be considered an antioxidant method (Chen, Cheng, & Liang, 2015). The method was first described in 1927 (Folin) and later modified and improved by Singleton and Rossi (1965). This method is simple, sensitive, and precise, and it can be read on a maximum wavelength of 765 nm spectrophotometrically (Chen et al., 2015). A gallic acid standard curve is used as a reference (Prior et al., 2005). Other chemicals have been used to make standard curves, but it is recommended to follow the method as strictly as possible as described by Singleton and Rossi (1965).

As for the DPPH method, the Folin method also offers some limitations, described by Box (1983); Peterson (1979); Prior et al. (2005). They mention several substances that can interfere, such as sugars, ascorbic acid, organic acids, and Fe (II), with possibilities to make corrections based on these interfering substances. A wide range of different non-phenolic organic chemicals and some inorganic chemicals can also interfere with the Folin reagent, resulting in elevated phenolic concentrations.

2.9.4 Vitamin C (HPLC)

The most investigated nutrient related to the processing and storage of food plants is vitamin C. Davey et al. (2000) explains this because it is both thermally labile and highly water-soluble, and losses during processing can occur due to ascorbic acid oxidase, thermal breakdown, and leaching of cells. The term vitamin C refers to both ascorbic acid (AA) and dehydroascorbic acid (DHAA) due to their anti-scorbutic effect, and they offer several health benefits (Nordic Council of Ministers, 2012).

Some common biological functions of AA results from its ability to inhibit and stop chain reactions of free radicals, thus acting as an antioxidant (Zhu, Tang, Tu, & Chen, 2020).

Vitamin C also takes part in the absorption of iron, synthesis of collagen, and reduction of cholesterol level in the blood (Tewari et al., 2017). There are also indications that it can prevent cancer (Valko et al., 2006). AA is converted to DHAA under oxidative stress, by losing two protons (see Figure 8). Nevertheless, DHAA can be recycled to AA by the enzyme dehydro-ascorbate reductase (DHAR) in different cells (Wilson, 2002). Further oxidation of DHAA results in diketogulonic acid, without any biological activity (Davey et al., 2000). The human body cannot synthesize vitamin C on its own, therefore it needs to be provided through the diet (Mazurek & Jamroz, 2015). Currently, the recommended daily allowance (RDA) of vitamin C for adult men and women is 75 mg/day (Nordic Council of Ministers, 2012).

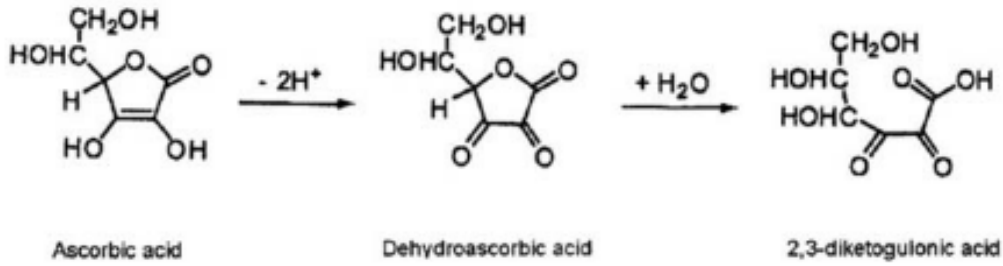

Figure 8. The oxidation reaction of L-ascorbic acid. Retrieved from Tewari et al. (2017).

The degradation rate of AA depends on the presence of oxygen, pH, presence of other antioxidants, thermal processing conditions, presence of metals, oxidizing lipid effects, presence of reducing substances, light, and ascorbic acid oxidase activity (Escobedo-Avellaneda et al., 2011). Due to these several factors, it is essential to use an extraction method that makes it stable before quantification (Chebrolu, Jayaprakasha, Yoo, Jifon, & Patil, 2012). To measure the content of AA, a common method is to use high-performance liquid chromatography (HPLC).

Nie and Nie (2019) describe the basic principles behind HPLC in detail and it is summarized below. The principle behind liquid chromatography is based on different adsorption or desorption abilities of components that are distributed in between two phases, the stationary and mobile phase. HPLC physically separates the different components of a sample, often in a solution, and gives information regarding the concentration of each separated component. In short, the system is composed of a solvent supply system (container for solvents and degasser), a high-pressure pumping system, a sampling system (injector), a chromatographic column (for separation), a detective system, and a data processing system. To provide a constant flow of solvents through the injector, column, and detector(s), the pumps need to generate high pressure. The reason is to overcome the resistance to flow in the column. Depending on what is to be analyzed, a wide range of columns can be used.

3. Materials and processing equipment

3.1 Raw material

Pieces of frozen cauliflower (*Brassica oleracea* var. botrytis L.) were obtained from Fjordkjøkken AS, and it was produced by Findus AS. The cauliflower pieces had been steamed blanched before freezing and were stored at -32 °C, and each batch had the same production date. A total of 4 different bags, each containing 15 kg were used for all experiments.

3.1.2 Treatment of raw materials before processing

Before packaging, the frozen material was stored in a freezer holding -32 °C. The cauliflower was weighed and vacuum-packed in several intervals to minimize thawing, followed by freezing 1 day before the treatment day. All experiments were done in triplicates. For shelf-life assessment, 5-10 g of cauliflower were weighed in separate bags. For measuring texture and color, 100 g were weighed in each bag. For the chemical measures, 210-250 g were weighed in each bag.

On the day of treatment, the vegetable packages were thawed in boxes filled with cold water at room temperature. After thawing, the packages were kept in ice-slurry before further processing. See Figure 9 below, illustrating how the thawing took place.

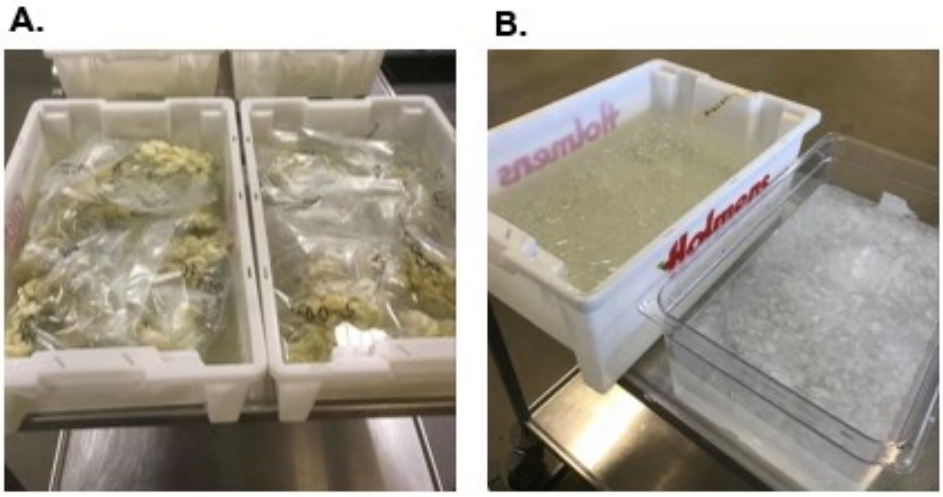

Figure 9. A, packages of cauliflower during thawing; B, boxes filled with ice slurry for rapid cooling.

3.2 Experimental design

The flowchart below (Figure 10) gives a schematic overview of the processing of cauliflower. In short, pieces of frozen cauliflower were packed and then thawed for a different duration depending on the amount being used for treatment. Then the bags of cauliflower were used for either high-pressure treatment (HPP), microwave heating (MW), or retort heating. For each treatment, a control (non-processed) of cauliflower was used for comparison. After treatment, the bags were cooled in ice-slurry, followed by refrigerated storage or freezing, until further analysis. Each process was repeated twice, with 3 processing replicates each time.

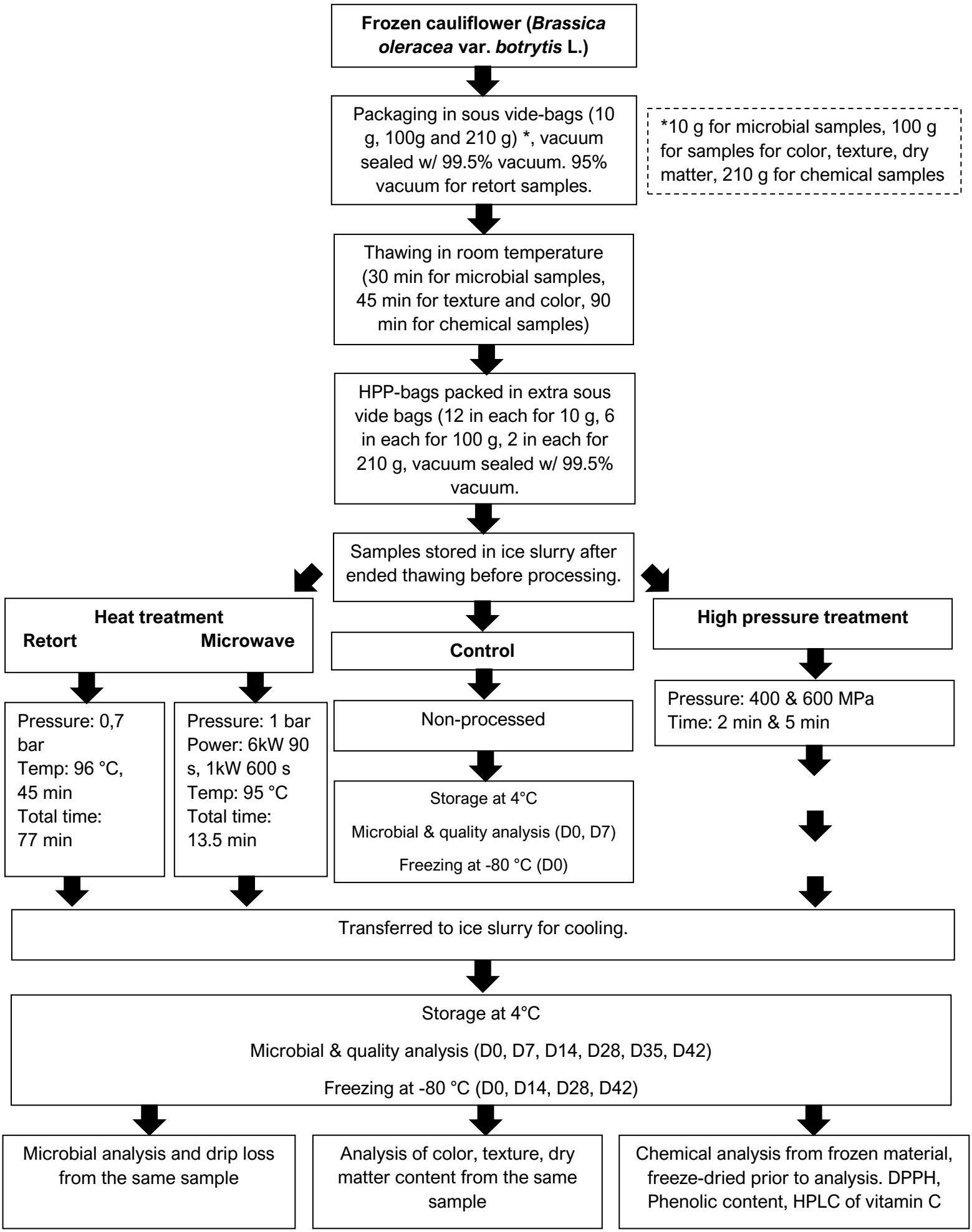

Figure 10. Schematic outline of the processing of cauliflower.

3.3 Processing Equipment

3.3.1 Vacuum packaging (VAC)

The cauliflower pieces were placed in an 80-µm standard sous-vide plastic bag (Arne B. Corneliussen AS, Oslo, Norway). The bags were vacuum packed (99.5% vacuum) in a vacuum packer (Webomatic SuperMax, Webomatic Maschinenfabrik GmbH, Bochum, Germany). Samples for retort treatment were sealed with 95 % vacuum, to avoid deformation of the tray.

3.3.2 High-pressure processing (HPP)

HPP was performed in a lab-scale high-pressure unit QFP 2L-700 (Avure Technologies Inc., Columbus, USA). The machine has a capacity of 2 liters and a 690 MPa maximum pressure level. Samples were pressurized at 600 MPa for 2 min or/and 5 min, and 400 MPa for 2 min or/and 5 min. In each operation, there was a come-up period, in which pressure is built up to the target pressure. The come-up time was approximately 90 s for 400 MPa and 120 s for 600 MPa. At the end of the holding period, the pressure was released immediately within seconds. Processing was at ambient temperature. The duration of treatment (2 min or 5 min) did not include the come-up time. Control samples were non-pressurized samples.

Before processing, all samples were vacuum-packed (99.5 %) in two vacuum bags, to avoid any potential leakage. After HPP, samples were immediately cooled in a box filled with ice slurry. The samples were stored at 4 °C until further analysis, for up to 42 days. Data from two different runs were obtained to compare and validate that the treatment was similar.

3.3.3 Microwave treatment (MW)

Vacuum-packed samples (600 g cauliflower in 6 individual vacuum-packed bags per batch of microwave operation containing 100-130 grams each) were heated in a laboratory microwave autoclave (Gigatherm AG, Flawil, Switzerland) operated at 2450 MHz. For each batch of processing, 6 bags were spaced approximately 1 cm apart and placed along the end of the rotating plate in the chamber, as shown in Figure 11. To verify the process conditions, 7 thermocouples were inserted in 1 dummy package with 100 g of cauliflower.

The pressure was sat to 1 bar above the atmosphere (resulting in 2 bar accumulating inside the microwave chamber). The power was introduced in two cycles, first 6 kW for 90 s, then 1 kW for 600 s (10 min), then a cool-down phase where the pressure was released, and temperature were sat to 0 °C, 1 kW, for 60 s. Then followed by 60 s for the total time it took for opening the valve and transferred the bags to ice slurry for cooling. After treatment, the samples were stored in a refrigerator at 4 °C

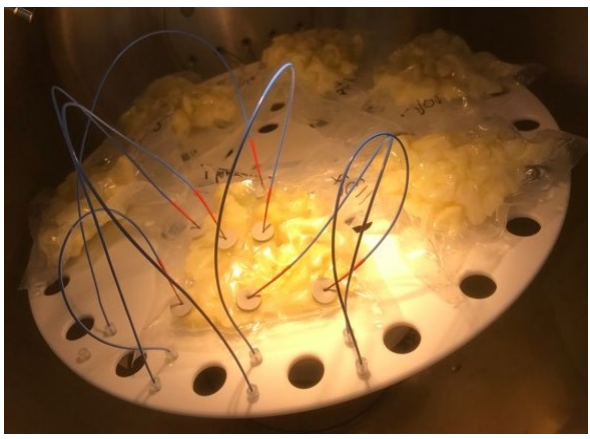

Figure 11. Shows the placement of the temperature probes in a bag of cauliflower before MW processing.

until further analysis. For the processing of cauliflower, a temperature of 95 °C was the target, with a reference temperature of 90 °C: $F_{90°C}$= 10 min, where the *z*-value was 10 °C for the target bacterium non-proteolytic *C. botulinum* type E. Data from 5 different runs were obtained to compare and validate if the treatment were similar.

3.3.4 Retort (conventional heat treatment)

The frozen cauliflower was packed in an oval black, single chamber tray (Færch, C 1203-1E, CPET plastic, depth 39,90 mm), thereafter sealed in a medium-sized 80-µm standard Sous-vide plastic bag (Arne B. Corneliussen AS, Oslo, Norway). For this treatment, the cauliflower for texture and color (100 g (+/- 20 g), chemical analysis (220-230 g), and for shelf-life study 18 separate bags containing small pieces of cauliflower (5-10 g) were packed together in one black tray. For microbial analysis were packed in a small Sous-vide plastic bag and packed together in one tray. This to ensure that they got a similar heat load as the other trays used for texture and chemical determinations.

Figure 12. A, the static retort; B, the opening of the valve; C, temperature measurement.

On the day of processing, the products were first thawed for 45 min in cold water at ambient temperature. Afterward, they were left for 30 min at ambient temperature without water for packaging in the boxes for the autoclave. A batch retort (Microflow 911 EAT, Steriflow, Roanne, France) was used. The packages were placed in a horizontal position in 3 sections (see Figure 12). The temperature profile was measured with a Type-T thermocouple connected to a data logger E-Val Flez (Ellab, Copenhagen, Denmark). To verify the process conditions, six thermocouples were inserted in three different dummy packages with 100 g of cauliflower. A total of two probes in each container were inserted in the stem of the cauliflower.

The retort system was heated by direct steam injection, equipped with a preheating tank to process water. This processed water was then circulated through a heat exchanger to the retort and spread by a perforated plate to obtain water raining over the products (trays with cauliflower). The total treatment time was 77 min. An extra 10 min were added before all the packages were transferred to the ice slurry. For the processing of cauliflower, a temperature of 96 °C was the target, with a reference temperature of 90 °C: $F_{90°C}$= 45 min, where the *z*-value was 7.5 °C for the target bacterium non-proteolytic *C. botulinum* type E. Data from two different runs were obtained to compare and validate that the treatment was similar.

The following program settings (Table 1) were selected based on industry-relevant conditions:

Table 1. The different temperature and pressure settings during the time of retorting.

Phase	**Temperature (°C)**	**Pressure (Bar)**	**Time (min)**
Heating	60.0	0.2	10.0
Heating	96.0	0.7	10.0
Heating	96.0	0.7	45.0
Cooling	60.0	0.7	2.0
Cooling	20.0	0.3	5.0
Cooling	20.0	0.2	5.0

3.4 Analysis of cauliflower

After different processing treatments, a shelf-life study for up to 42 days was conducted, with sampling days 0, (7), 14, 28, (35), and 42. On days 7 and 35, only microbial analysis and drip-loss were assessed. The untreated samples (control) were analyzed at day 0 for all analysis, and microbial analysis also at day 7 (only for total aerobic counts). The samples were either used for measuring texture and color or frozen and kept at −80 °C until chemical analyses. The same samples (n=3) used for color were also used for texture analysis afterward. After texture analysis, the dry matter was analyzed with the same samples.

All analyses except chemical were performed directly after processing and storage. Samples for chemical analysis were taken out from refrigerated storage for each day (0, 14, 28, 42) and frozen at −80 °C, then shipped to Trondheim at −32 °C. Further, all samples were freeze-dried (Labconco, FreeZone 12) at 0.12 mBar for 36 hours and milled before analyses.

3.4.1 Microbial shelf-life

The microbial flora of processed cauliflower was determined after processing (day 0) and after refrigerated storage (day 7, 14, 28, 35, & 42). Microwave treated samples were only analyzed until day 14. The cauliflower (5-10 g) was placed in a sterile filter bag (Separator 400 Blender Bag, Grade Products Ltd., Leicestershire, UK) with 90 mL (diluted 1:10) of 1 % sterile peptone water (8.5 g/l NaCl and 1g/l bacteriological peptone, Oxoid) and smashed for 2 min using a stomacher (Smasher, BioMerieux Industry, MO, USA) at the fast speed setting. The filtered liquid was transferred to 15 ml Falcon tubes, and then serially diluted with peptone water and spread onto plates of Plate Count Agar (PCA) (Merck-Millipore Corp., Billerica, USA). Measurements were made in triplicate per sample treatment. The suspensions were plated on PCA for detecting viable cells of total aerobic bacteria (TAB) (colony forming units [cfu] per g). The plates were incubated at 37 °C for 48 hours.

For detecting viable aerobic and anaerobic spores, 5 ml samples of the stomacher solution were heated in a water bath for 10 min at 80°C in water bath to inactivate vegetative cells. Thereafter the tubes were cooled to room temperature for 5 min in cold water and plated on PCA. The plates were incubated at 37 °C for 48 hours (+/- 3 hours). Anaerobic spores were incubated in an anaerobic box with an indicator. The presence of spores was checked at day 0 (control samples), and day 35 and 42.

A mechanical spiral plater (Eddy Jet, IUL Instruments, Barcelona, Spain) was mainly used for all enumerations (49.2 µl injection volume), but some manual plating (500 µl) was performed for low dilutions. The detection level on PCA was 2.3 log cfu/g. For plates with no colonies detected, the level was set to half of the detection limit (as described by Aaby, Grimsbo, Hovda, and Rode (2018)). After incubation, all colonies on plates were counted. The results were calculated and given as log cfu/g sample. For statistical analysis convenience, when samples showed no microbial growth a value that was half of the detection level was used. The microbial counts are the mean of 3 experiments (pilot experiment + 2 main experiments).

3.4.2 Color

The color of both stem and floret of the cauliflower was determined before and after processing, and during each day of sampling (day 0, 14, 28, 42), using VeriVide's DigiEye system (VeriVide Ltd., Leicester, UK). This system was equipped with a DSLR camera (Nikon D90, Japan) and it captured an image of 4288×2848 pixels with a resolution of 96 dpi.

Before each measurement, the camera was white balanced and calibrated with the color chart provided with the equipment. The L*, a*, and b* components were recorded at a D65 standard illuminant. Each piece of the sample was photographed in a light cabinet on a black background, as can be seen in Figure 13.

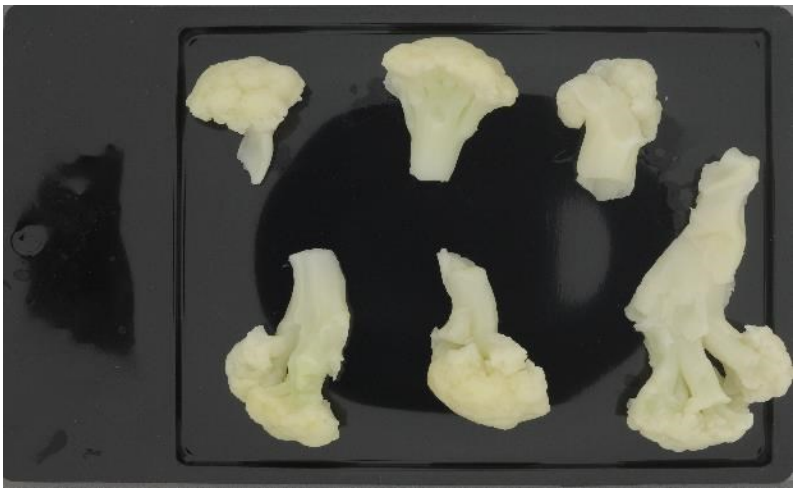

Figure 13. Shows how the pieces of cauliflower (HPP 400 MPa/2min, day 0) were placed on a black tray before capturing the image.

The captured images were analyzed for CIELAB color scale using the DigiEye 2.9 software, with the use of the «free form tool» because of the irregular shape of each piece. Data were obtained from 18 measurements on each sample.

The L^* a^* b^* color space was used for the determination of the color. Further, the total color difference (TCD) of the samples before (control) and after processing and storage were calculated as:

$$\mathrm{TCD} = \sqrt{[(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]}$$

where L_0^*, a_0^*, and b_0^* were the values for the untreated sample (control).

3.4.3 Texture

The firmness of the cauliflower was measured before and after processing, and during each day of sampling (day 0, 14, 28, 42). A Texture Analyzer XT Plus (Stable Micro Systems Ltd., UK), with a TA-42 knife probe with 45° chisel blade, guillotine block, and slotted plate were used (see Figure 14). The method applied was as described by Koskiniemi, Truong, McFeeters, and Simunovic (2013), with some modifications. This type of test was used to quantify the force and the amount of work required to shear through the entire sample. A 50 kg load cell was used for all texture measurements. The pre-test speed was 3.00 mm/s, the test speed was 2.50 mm/s, and the post-test speed was 10.00 mm/s. The test mode was compression. Further, the trigger force was set to 6 *g*, and the measured force was applied at 50 % strain. Both height and weight were calibrated before analysis. All samples were tempered at room temperature for 1 h before analysis.

Figure 14. Arrangement of 3 pieces of cauliflower during texture analysis.

For each measurement, three cauliflower stems (2-3 cm in length and 0.5-1.0 cm in diameter) were placed perpendicular over the slot of the guillotine holder, and the knife probe cut all the way through the vegetable material and advanced through the slotted plate. The tests were performed in duplicate for each bag of cauliflower sample. For each treatment, a total of 6 samples were measured per day of sampling. The texture property «firmness» (hardness) was defined as the positive peak force (cycle 1) from the resulting curve of the first compression of the sample because it mimics the first bite. The maximum force (*kg*) required to shear the sample was measured.

3.4.4 Drip loss

The drip loss of cauliflower was measured before and after processing, and during each day of sampling (day 0, 7, 14, 28, 35, 42). For the HPP and retort treatment, 5-10 g individual pieces were used. For the microwave treatment, the drip loss was weighed from 100 g of cauliflower. This was due to the difficulty of processing small individual pieces in the microwave oven (resulted in an expansion of the package, and that the thermocouple was ejected). The drip loss was calculated according to the formula below:

$$Drip\ loss\ (\%) = \frac{Weight\ at\ packaging\ (g) - Weight\ at\ sampling\ day\ (g)}{Weight\ at\ packaging\ (g)} \times 100$$

3.4.5 Dry matter

The dry matter content of cauliflower was measured before and after processing, and during each day of sampling (day 0, 14, 28, 42). For measuring the dry matter content of cauliflower, 5 - 8 g of non-processed (control) and processed samples were weighed in aluminum trays, then heated at 105 °C for 16 - 18 hours. After heating, the samples were stored in a desiccator for 30 min before weighing. The formula used for measuring the water-, and dry matter content is the following:

$$Water\ (\%) = \frac{Wet\ weight\ (g) - Dry\ weight\ (g)}{Wet\ weight\ (g)} \times 100$$

$$Wet\ weight\ (g) = Weight\ of\ tray\ (g) + Weight\ sample\ before\ drying\ (g)$$

$$Dry\ weight\ (g) = Weight\ of\ tray\ (g) + Weight\ sample\ after\ drying\ (g)$$

$$Dry\ matter\ content\ (\%) = 100\ \% - Water\ (\%)$$

3.5 Chemical analysis

The differently treated cauliflower samples were taken out from cold storage (4 °C) after each day of sampling (day 0, 14, 28, and 42), and frozen at -80 °C. Thereafter, the samples were sent to Trondheim at -32 °C, followed by freeze-drying (Labconco Freezone 12) in several batches. After drying the samples were milled and stored at -32 °C until further analysis. Each experiment except for ascorbic acid (HPLC) was repeated three times.

3.5.1 Total antioxidant activity (DPPH)

The antioxidant activity levels of the freeze-dried cauliflower were determined according to their 2.2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging activity, as described by Ahmed and Ali (2013) with some modifications. First, a methanolic solution of 0.1 mM DPPH was made and stored cool in a dark bottle at 4 °C. Then, 0.5g of freeze-dried powdered cauliflower was added to 15 ml tubes, and 10 ml of methanol was added in each. After, the mixture was homogenized (IKA T25, Ultra Turrax) for 1 min, followed by centrifugation at 5000 rpm for 10 min (Eppendorf centrifuge 5804). This supernatant was also used for phenolic content determination later.

Further, 0.1ml of the aliquot was added to a lab tube, and 4 ml of the DPPH solution was added. The absorbance of the DPPH reagent was determined after incubation for 30 min in dark, using a spectrophotometer (Shimadzu UV-1800) set at 515 nm, by measuring the sample absorbance decrease against a negative control. The DPPH radical scavenge activity results in decolorization and were calculated in terms of percentage reduction of DPPH according to the following equation:

$$Radical\ scavenge\ activity\ (\%) = \frac{C - A}{C} \times 100$$

where *A* is the difference in absorbance at 515 nm between control and sample, and *C* is the absorbance of the control. For control samples, 0.1 ml of methanol was used instead of the extract. Analyses were performed in duplicate on each sample, and results are the mean of three repetitions of the experiment.

3.5.2 Total phenolic content (TPC)

The total phenolic content of the vegetables was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965), with modifications from Ahmed and Ali (2013). The methanolic extracts from the DPPH experiment were used here also. Briefly, 0.1ml of the cauliflower extract was transferred to a 15 ml plastic tube, and 7.9 ml deionized water was added. Afterward, 0.1 ml of Folin-Ciocalteu phenol reagent were added to each sample tube and left for 3 min. Then 2 ml of 20 % of sodium carbonate were added and vortexed. Samples were left for 60 min in dark, and absorbance was measured at 760 nm. For control samples, 0.1 ml of methanol was used instead of the extract. Various concentrations of gallic acid standard solution were used to establish the standard calibration curve. The results were expressed as mg of gallic acid equivalents

(GAE) per 100 g of dry weight (DW). Analyses were performed in duplicate on each sample, and results are the mean of three repetitions of the experiment.

3.5.3 Ascorbic acid (AA)

Analysis of ascorbic (AA) acid in the extracts (20 µL injection volume) was performed on an Agilent 1100 Series HPLC system (Agilent Technologies), with modifications of the method described by Castro et al. (2008). Before extraction and HPLC of the cauliflower, a buffer was made by mixing 27.2 g of KH_2PO_4 in 30 mL methanol, then adding 900 ml of deionized water. The pH of this solution was adjusted with phosphoric acid to a target pH of 2.4, to prevent degradation of ascorbic acid. Before adding an HPLC eluent, the solution was filtered. For the preparation of standard ascorbic acid, this buffer was used as a solvent. A stock solution of 500 µg/ml was prepared, with 25 mg for 50 ml.

For the extraction of ascorbic acid, 80 mg of powdered cauliflower samples were dissolved in 10 ml of 4 % metaphosphoric acid in deionized water. This solution was homogenized for 2 min and rotated for 20 min in dark. After, the mixture was centrifuged at 5000 rpm for 10 min (Eppendorf centrifuge 5804), then filtered using a filter syringe and transferred to HPLC vial.

Separation was performed at room temperature on a monolithic column with mobile phase 0.2 M KH_2PO_4 adjusted to pH 2.4. The retention time for AA was approximately 4.37 min, with a flow of 0.800 ml/min and injection interval were 7 min. Further, the AA was detected at 254 nm and quantified by an external standard. Each sample was analyzed in triplicate. The identified ascorbic acid (254 nm) was quantified based on their peak area and compared with calibration curves obtained with the corresponding standards and then expressed as mg AA/100 g DW.

3.6 Statistical analysis

Statistical analysis was performed using Minitab® Statistical Software (version 19, Minitab Ltd., Coventry, UK). Significant differences ($p<0.05$) between samples throughout processing and storage (processing replicates; $n = 3$) were determined with one-way analysis of variance (ANOVA) and *posthoc* test Tukey's multiple comparison test. General linear modeling ANOVA was performed to determine significant effects of the experimental factors and their interactions.

4. Results and discussions

In this section, the different results from the quality analysis of cauliflower are presented and discussed. This thesis aimed to investigate the potential for HPP and MW processing of cauliflower and how this technology affects the quality compared with conventional thermal processing.

4.1 Microbial shelf-life

Total aerobic bacteria (TAB) were determined in cauliflower before and after processing and storage (see Table 2). Pressurization of cauliflower at 600 MPa for 2 and 5 min reduced the TAB by 2.0 and 2.3 log cfu/g, respectively, at day 14. HPP at 400 MPa for 2 and 5 min had low counts until day 7, but high counts at day 14 (6.4 and 6.1 log cfu/g, respectively). The non-treated samples (control) showed bacterial counts of 5.0 log cfu/g at day 0. The HPP holding time did not significantly ($p>0.05$) change the bacterial counts at all days (except 400 MPa day 0), indicating that shorter processing times could be beneficial. Throughout the storage, and especially at the end of the storage period a larger deviation between the samples was found. Some individual 600 MPa samples had no detectable growth at day 42, whereas others had high counts. The pressure of 600 MPa had a significant ($p>0.05$) effect on TAB. The increase in TAB could be due to the growth of surviving cells, in addition to a recovery of injured cells.

The control samples varied significantly from the other samples. When looking at the pressure applied for 400 and 600 MPa until day 28 of storage, there was a significant difference between the two ($p<0.05$) pressures. This supports the hypothesis that higher pressure leads to less microbial activity when it comes to TAB. When processing at 400 MPa for 2 or 5 min a shelf-life between day 7 and 14 could be estimated.

Retorted samples had no visible growth of TAB and remained below detection level (2.3 log cfu/g) until day 42, indicating good preservation by the heating. Both runs of retorting resulted in a similar heat load (see appendix A), with a calculated average F-value of 163 min. All the packages for both runs held a temperature of 90-96 °C for 45 min. This could indicate a possible inactivation of Non-proteolytic *C. botulinum* type E if present. To validate the possible inactivation of this pathogen with the selected processing conditions, the cauliflowers could have been inoculated with an indicator bacterium to more precisely determine this. The processing parameters were selected from the industrial partner for this research since they used 96 °C for their ready-to-eat dish. Consequently, by only heating the cauliflower with these conditions, a different heating distribution was obtained than in the RTE meal product.

Table 2. Microbial counts, TAB (total aerobic bacteria [log cfu/g]) * in cauliflower before (control), and after HPP, microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

Storage (days)	Control	HPP				Retort	Microwave
	0.1 MPa	400 MPa		600 MPa		96 °C	320 kJ
	0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
0	5.0±0.2$^{A, a}$	3.3±0.2$^{A, b}$	<2.3 $^{A, c}$	2.4±0.4 $^{A, d}$	<2.3 $^{A, d}$	<2.3 $^{A, d}$	3.6±0.5$^{A, b}$
7	7.2±0.9 $^{B, a}$	3.8±0.3 $^{B, c}$	3.2±0.3$^{A, c}$	<2.3 $^{A, d}$	2.3±0.3 $^{A, d}$	<2.3 $^{A, d}$	5.6±0.9 $^{B, b}$
14	nm**	6.4±0.5$^{C, b}$	6.0±0.7$^{B, b}$	3.1±1.0 $^{A, c}$	2.8±0.4 $^{AB, cd}$	<2.3 $^{A, d}$	8.2±0.4$^{C, a}$
28	nm	9.3±0.5$^{D, a}$	9.1±0.5$^{C, a}$	5.5±1.7$^{B, b}$	5.0±1.9$^{BC, b}$	<2.3 $^{A, c}$	nm
35	nm	nm	nm	6.6±1.9$^{B, a}$	6.1±2.7$^{CD, a}$	<2.3 $^{A, b}$	nm
42	nm	nm	nm	5.7±2.8$^{B, a}$	7.5±2.2 $^{D, a}$	<2.3 $^{A, b}$	nm

* Each TAB value in the table is the mean ± standard deviation from processing parallels (n=3). The capital letters (A, B, C, and D) indicate a significant difference ($p<0.05$ multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, c, and d) indicate a significant difference ($p<0.05$) between different treatments and days (same row). The detection level was 2.3 log cfu/g. Where no survival was detected, the values were set to 50% of the detection limit (log 2.0).

**Not measured.

The MW samples had high bacterial counts (5.6 log cfu/g) already at day 7, and they were spoiled (8.2 log cfu/g) at day 14, indicating that the process conditions were not optimal, with too low heating. A likely source of error was that the pieces of cauliflower varied in size, thus the microwaves could have penetrated differently in each resulting in uneven heating and cold spots where microbes survived. Another uncertainty was the start temperature. The biggest pieces could have been close to frozen after thawing. Thawing in running water could shorten the thawing time and provide a more even start temperature. Also, pieces of cauliflower have large surfaces where possible bacteria could survive during processing.

When looking at the SD of the different samples, some are quite high. The high SD for 600 MPa at the last sampling days (35 and 42) can be explained as some of the samples had high growth, whereas others had no growth (under detection limit). For instance, 2 of 3 bags had no visible growth for one of the experiments at day 42 (600 MPa, 2min). According to van Schothorst, Zwietering, Ross, Buchanan, and Cole (2009), an SD of 0.80 log cfu/g for an inhomogeneous food (e.g., solid food) should be expected. They further mention that in some cases larger inhomogeneity could occur, for instance, if there has been surface contamination. If the different cauliflower pieces came from different harvest locations, they could have had differences in microbial load, and this could lead to variation in the results.

Proteins and membranes are the primary targets for the pressure-induced inactivation of bacteria (Ulmer, Ganzle, & Vogel, 2000). Pressures above 400 MPa affect vegetative bacteria in several manners by disturbing cellular physiology (metabolism, cell membrane physiology, transport, and gene-expression (Vogel et al., 2003). With some exceptions, pressure resistance is higher in Gram-positive bacteria than in Gram-negative

bacteria, because Gram-positive has a thick peptidoglycan layer (Arroyo, Sanz, & Prestamo, 1999; Arroyo et al., 1997). For further research, the effects of specific strains of bacteria in cauliflower would be interesting to look at. Bover-Cid, Belletti, Garriga, and Aymerich (2012) claims that as a cold pasteurization process, HPP aims to reduce loads of pathogenic microorganisms of approximately 4 to 6 log.

The maximum temperature in the high-pressure chamber was dependent on the set pressure, which for 400 and 600 MPa were 29 and 33 °C, respectively. The difference in temperature during processing and potential differences in thawing temperature could lead to some variation of the microbial counts. Perhaps, the temperature increase caused by the compression of HPP heating could have resulted in a slight thermal effect, which could contribute to the lethality of some bacteria. Heinz and Buckow (2009) mention that pressure stability of vegetative microbes usually decreases at lower temperatures, therefore it could be positive that the cauliflower was processed directly after thawing.

The HPP samples had an acidic smell after day 28, indicating possible lactic acid bacteria present. Vegetables have a low buffering capacity, which makes the conditions favorable for lactic acid bacteria to grow during anaerobic conditions (Fung, 2009). Measures of the pH during storage could also indicate spoilage. Ulmer et al. (2000) investigated the effects of pressure on *Lactobacillus plantarum* in beer, a possible spoilage bacterium. They found that at pressures of 600 MPa, all cells were killed during pressure and the fraction of barotolerant (pressure tolerant) cells was at or below the detection level (120 cfu/ml). They concluded that the use of HPP was effective in preventing or delaying the growth of spoilage bacteria in beer, instead of using sterilization with high temperature. The carbohydrate content of cauliflower could contribute to some protection of pressure in bacteria (Dong et al., 2013).

Few studies have been conducted on the effects of HPP on the shelf-life of cauliflower, and none during storage of 42 days. Among the few, Arroyo et al. (1999) looked at the effects of HPP of cauliflower (300, 350, 400 MPa/5 °C/30 min) on different microbes. They found low counts (below detection limit) of TAB at 400 MPa immediately after processing. Nevertheless, they processed their cauliflower in homogenized pieces in peptone water. This is different from whole pieces, and concerning the long processing conditions, comparison of their results is difficult. Arroyo et al. (1997) tested TAB on HPP of tomato and salad (100-400 MPa/20 °C/10 min or 10 °C/20 min). They found that 400 MPa gave the highest reduction of bacterial counts, but complete inactivation was not achieved.

Chen, Guan, and Hoover (2006) found a linear inactivation relationship with the increase of pressure from 0 to 690 MPa for different pathogens in milk. In some cases, they found small populations of *L. monocytogenes* were very resistant to high pressures. They explained that pressure sensitivities could vary within populations, possibly due to different physiological states. The authors also point out that different test

conditions make a comparison between studies difficult, as pressure resistance will vary with the type of substrate the microbes are in.

Dong et al. (2013) tested the effects of HPP (400 MPa/ 10 min and 600 MPa/2.5-5 min) on TAB for lotus root during 3 months of cold storage (4 °C). Their results confirm our results for cauliflower, that 600 MPa gave the lowest bacterial counts. Directly after processing, 600 MPa for 5 min gave 1.94 log cfu/g. After 14 days of storage, the authors got a TAB of 2.23 log cfu/g, while the TAB remained lower than 3.14 log cfu/g for up to 90 days (600 MPa, 2.5 min). For 400 MPa they reported 4 log cfu/g at day 30. They reported high SD after 60 days of storage. This type of microbial stability could not be observed for cauliflower in this study since high counts were observed for 600 MPa at days 35 and 42.

For the MW treatment, a target of 95 °C for 10 min was based on the 6 log reduction of Non-proteolytic *C. botulinum* type E, and 6 kW was chosen to give a rapid temperature rise. According to Peng et al. (2017), a temperature less than 85 °C is not enough to give a 1 log reduction of this bacteria after several min of heating. They further mention that 1 min at 70 °C can be sufficient for a 1 log inactivation for vegetative bacteria. For inactivating *Bacillus cereus*, they point out that higher temperatures above 95 °C are needed. Unfortunately, the coldest spot in some of the MW cauliflower did not receive temperatures above 80 °C. This resulted in low calculated F-values for the target bacterium (0.0031 min on average), indicating insufficient heating (see appendix B). An example of a typical temperature profile can be seen in Figure 15, with the coldest point in the middle of the package.

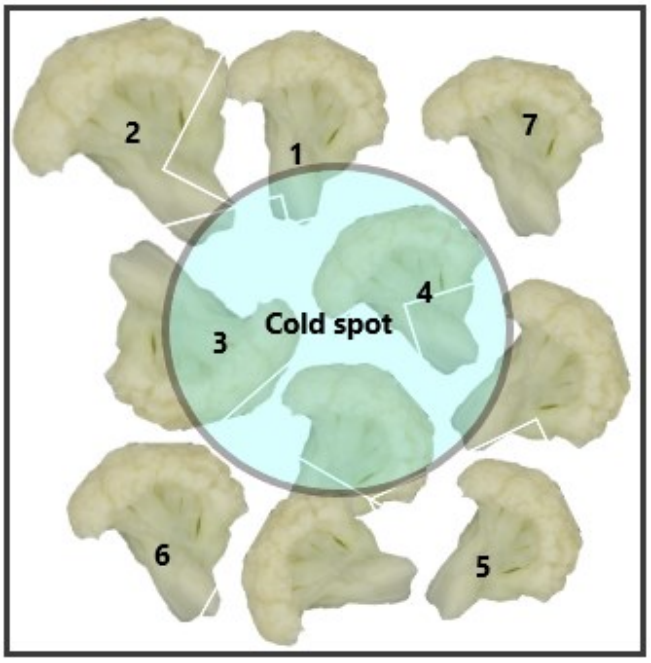

Figure 15. An example of the temperature and placement of probes (1-7) during run nr. 5 of MW treatment. The temperature of the different probes after 11 min of heating was the following: (1) 80.8 °C; (2) 83.7 °C; (3) 74.4 °C; (4) 77.9 °C; (5) 90.7 °C; (6) 95.

At day 0, a 1.42log inactivation was achieved for the MW cauliflower, compared to the control. However, a different sampling procedure was used for the MW samples than for the other processes. This was a result of difficulties with treating single vacuum-packed pieces (10 g) of cauliflower since the package expanded too much. Therefore, for the microbial analysis, it was decided to take out small pieces from a 100 g package of cauliflower, with two pieces from the middle of the package (cold spot) and two pieces of the outer edges, followed by homogenizing and colony counts. Nevertheless, since sampling was taken from several pieces of the bag, this could result in some insecurities. Further, the plastic around some of the packages had melted, and this could result in the release of vacuum, leading to possible contamination of samples. To avoid this an extra surrounding packaging after MW treatment could have been used.

Several possible sources of variation for MW can describe the uneven heating. As mentioned in section 2.5.1, the dielectric properties vary from frozen and thawed foods. Since not all pieces of cauliflower were properly thawed, this is likely to result in variation of MW heating distribution. When the temperature increases in some pieces, the dielectric properties also change, thus the warmest parts will absorb most of the MW energy (Tang, 2015). In other words, when a temperature difference has occurred, this difference will increase even further between the coldest and warmest parts. When inserting the thermo-probes, the vacuum of the dummy package was released, thus more steam and heat could evaporate, while the other packages could get a higher temperature. Also, further processing of the bags for a longer duration could lead to overheating of the outer parts of the cauliflower. In this way, the middle part of the package could retain a consistent quality, while the outer parts got overcooked. From a consumer perspective, this would not be appropriate. Therefore, further processing conditions are needed to find an optimal treatment that results in appropriate shelf-life. The amount of cauliflower in each bag was the same, and the distance between each bag was close to similar, but the size of the pieces varied. The inhomogeneous pieces of cauliflower made it difficult to standardize the MW process in advance. Vadivambal and Jayas (2010) highlight the importance of uniform thickness of MW food to be maintained whenever possible, and that control of geometry is essential for improved heating. The nature of cauliflower makes it difficult to standardize, but a suggestion could be to minimize the pieces further by cutting before use.

The heating of MW of foods is still not fully understood, and one of the reasons for the slow industrial application. Vilayannur, Puri, and Anantheswarana (1998) used potato as a model food for MW heating and found that, for brick-shaped products, hot spots were at the corners, while cold spots were located at the geometric center. This could also show some resemblance to the cauliflower package, that had a squared size. Ryynänen and Ohlsson (1996) measured the heat distribution for an RTE meal (mashed potato, carrots, meat, and sauce), and found that a cool-down phase of 5 min resulted in an even heat distribution due to heat conduction. They further suggested the need to use several methods of measuring the temperature, to get more reliable data. The

authors also explained edge heating with an antenna effect of the microwaves because foods with edges can possibly center the waves. This could possibly be one of the reasons for the outer hot spots for the cauliflower packages.

It is difficult to compare results from MW cooking of cauliflower with other studies due to the high variability of the method used for heating (different time, temperature, and power (kW), placement of the vegetable, condition, some uses frozen material, etc.) Irregular-shaped products are subjected to nonuniform heating due to the difference in product thickness, and this is likely the case when using cauliflower. To get a better understanding of how microwaves behave in cauliflower, it could be possible to measure the dielectric properties. At the same time, this is complex and depending on a specific temperature. Some limitations in this measurement are that air gaps, due to rough and fibrous surfaces in vegetables, could strongly affect the performed measurements (Angela & Damore, 2012).

Since MW alone did not reduce bacterial counts sufficiently, possible combinations with other treatments could be an option, giving possible synergistic effects. For instance, Yuan, Lu, Koutsimanis, Ge, and Johnson (2018) found that HPP (378 MPa/3 min) followed by MW (20 W/g/ 35 s) yielded a 4.33 log cfu/g reduction in bamboo shoots. They also found that the holding time of pressure was less significant than other parameters and suggested improved efficiency due to this. This was also the case for this experiment, where no significant difference ($p>0.05$) was found between 2 or 5 min applied at both pressures. Another possible combination is MW pre-treatment followed by mild retorting. Cho and Chung (2020) found that microwave pre-treatment of vegetables before retorting improved the quality because milder retort conditions could be used.

Total bacterial counts are a general analysis used as an indication of the shelf-life of foods, but a more detailed analysis of bacterial flora present is needed. It would be interesting to further investigate the specific type and strains of bacteria present in the processed cauliflower. The detection of possible spores in the food could also provide more info when assessing the shelf-life.

4.1.2 Aerobic and anaerobic spores

Bacterial spores represent a hazard for the food industry first when they germinate, and grow in the food, which can lead to food poisoning and spoilage (Heinz & Buckow, 2009). For both aerobic and anaerobic spores, low growth can be detected for all samples, at all stages during the storage period (Table 3). No spores were detected at day 0 of the control sample. The presence of aerobic and anaerobic spores was also checked on day 35 and 42 for HPP and retort. For HPP, no significant differences were found both within or between treatments and days ($p>0.05$), and most counts were close to the detection limit. All counts for retort were below the detection limit (2.3 log cfu/g), indicating successful heating.

Table 3. Microbial counts (total viable count [log cfu/g]) * for aerobic (A) and anaerobic (B) spores in cauliflower before (control), and after HPP, microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

A. Aerobic spores					
Storage (days)	**Control**	**HPP**		**Retort**	**Microwave**
	0.1 MPa	**600 MPa**		**96 °C**	**320 kJ**
	0 min	*2 min*	*5 min*	*45 min*	*10 min*
0	<2.3	nm	nm	nm	nm
35	nm**	<2.3	2.4±0.9	<2.3	nm
42	nm	2.4±0.8	2.6±1.1	<2.3	nm
B. Anaerobic spores					
	Control	**HPP**		**Retort**	**Microwave**
Storage (days)	**0.1 MPa**	**600 MPa**		**96 °C**	**320 kJ**
	0 min	*2 min*	*5 min*	*45 min*	*10 min*
0	<2.3	nm	nm	nm	nm
35	nm	2.7±1.1	2.6±0.8	<2.3	nm
42	nm	2.5±0.7	2.6±1.14	<2.3	nm

* Each TAB value in the table is the mean ± standard deviation from processing parallels (n=3). The detection level was 2.3 log cfu/g. Where no survival was detected, the values were set to 50% of the detection limit (log 2.0).

**Not measured.

Even low counts of spores could pose a threat to food safety. With the right conditions, the spores present in the cauliflower could germinate and cause food poisoning. It is therefore of utmost importance to apply more specific methods to detect viable spores after processing. Unfortunately, bacterial spores are extremely resistant to commercially attainable pressure levels. In this manner, low-acid shelf-stable products cannot be achieved by elevated pressure only (Vervoort et al., 2012). Since cauliflower is a low acid product, HPP might not alone make it safe to eat, even at 600 MPa. Pressures up to 1000 MPa has been suggested to inactivate spores sufficiently, but from an industrial point of view, this would demand higher energy output and more time to build up pressure (Borch-Pedersen et al., 2017).

Since these mild conditions are insufficient to achieve bacterial spore inactivation, units operating at higher pressure (600–800 MPa) and elevated temperature (90–120 °C) could be necessary (Serment-Moreno et al., 2014) This combination of high pressure and high temperature has shown to be successful in reducing the bacterial spore load (Ates et al., 2016; Gao et al., 2011; Reineke et al., 2013). Nevertheless, the different food matrixes that have been looked at do not involve pieces of cauliflower. The food matrix could affect how well bacteria survive, where large pieces with several gaps can be favorable conditions to survive in.

The overall picture showed that HPP gave low growth of spores. Nevertheless, this could also be a result of cold storage since spores grow slowly in these conditions. A

hypothesis could be that lactic acid bacteria have reduced the pH to make the conditions unfavorable. A measure of pH should have been done in this case. Since the pieces were vacuum packed in anaerobic conditions, this could favor the growth of lactic acid bacteria.

Arroyo et al. (1997) tested different pure cultures of microbes and vegetables (tomato and salad) on HPP (100-400 MPa/20 °C/10 min or 10 °C/20 min). They found that reductions of cultures of *Bacillus cereus* spores at 400 MPa (10 °C/20 min) gave less than 1 log cfu/ml reductions. Further, Arroyo et al. (1999) found that strains of *Bacillus cereus* spores (in pure suspension) were sensitive to 400 MPa, (>10^2 cfu ml). They also used flow cytometry to detect viable and dead cells of this bacteria and found 23.7 % remaining living cells after 400 MPa.

The method applied for counting anaerobic spores had some limitations, and this could make the results less reliable. According to Fung (2009), samples need to be introduced into the melted agar to provide optimal conditions for anaerobic growth. This was not done for this research. The author also mentions that during the time as gas packs make the environment anaerobic, some strictly anaerobic microbes could die. Regarding this, it could be necessary to use another method for detection.

4.2 Color

Color is a useful criterion of quality and can indicate deterioration during storage (Vervoort et al., 2012). The total color difference (TCD) data for both the floret and stem of cauliflower are summarized in Table 4. TCD is a relative difference in the L*, a*, b* values compared to a non-treated sample (control), where a value above 3 is possible the detect by the human eye. The individual L*, a*, b* values can be found in Appendix C. In general, the TCD was higher for the floret than the stem, one reason could be shadows from the floret, causing lower L*-values. All HPP florets also had significantly higher ($p<0.05$) b*-values during storage compared to the stem, indicating more yellow colors.

Overall, both samples processed at 400 and 600 MPa for 2 and 5 min gave low changes in TCD during storage. At day 0 the stem of HPP samples was below or close to a TCD of 3, indicating low changes from the control sample. There was not found a significant interaction between pressure and time during HPP on the TCD of both stem and floret ($p>0.05$). For the stem within 400 MPa (2 min), only day 42 is significantly different from day 0 and 14 ($p<0.05$). Samples treated with retort were significantly different to both control and the HPP samples at days 14, 28, and 42 ($p<0.05$). The high TCD value for the stem of the retort samples until day 42 was due to low L*-values (from 76.8 to 71.5) and increased a*-values (-0.9. to +0.7), indicating darkening and less green color of the sample.

Miglio, Chiavaro, Visconti, Fogliano, and Pellegrini (2008) & Oey et al. (2008) explained that differences in color during HPP could be related to textural changes, where the

loss of firmness could change the surface reflecting properties and light penetration, yielding changes in lightness (L*-value). Microbial deterioration during storage could also affect the color measurements, such as sliminess (Trejo Araya et al., 2009). Alvarez-Jubete, Valverde, Patras, Mullen, and Marcos (2014) found an inverse correlation with texture values and changes in TCD of HPP white cabbage (400/600 MPa, 20/40 °C). This could explain the changes in TCD of HPP cauliflower also. Trejo Araya et al. (2009) also found that the color of HPP carrots (600 MPa/2 min) was well preserved during storage (4 °C) for up to 14 days. These findings agree with those of Vervoort et al. (2012), who reported a TCD of 2.52 on HPP carrots (600 MPa, 10 °C, 10 min).

As can be seen, retort treatment gives a significantly higher TCD ($p<0.05$) compared to the other samples. High-temperature treatment of vegetables can cause enzymatic browning due to reaction with the enzyme polyphenol oxidase (PPO) and peroxidase (POD). Both enzymes can through several reactions produce brown-colored pigments in food (Billaud et al., 2005). Possible residual activity of lipoxygenase could also explain the change in color since 100 °C is needed to fully inactivate it. Residual enzyme activity should be measured for the different treatments to confirm this, as enzymes could explain other quality parameters related to texture and nutrition as well.

For MW samples, the floret had a low TCD at day 0, while significantly increased at day 14 ($p<0.05$) possibly due to enzymatic and microbial deterioration. Pellegrini et al. (2010) subjected both fresh and frozen cauliflower for MW (0.3 kW) for 30 and 20 min, respectively. They found low values of TCD for frozen (2.1) and fresh (4.0) cauliflower. The authors explained the low TCD of frozen cauliflower with the color stabilizing effect of blanching before freezing.

The different pieces of cauliflower shown in Figure 16 visualize the color changes during storage. On day 0, a fresh appearance can be seen in all samples (included HPP at 5 min) except the retorted. This is partly due to the greenness in terms of negative a*-value, where retort samples had a significantly different (higher) a*-value ($p<0.05$). The HPP samples got a paler color towards the end of storage, with an increase of negative a*-value indicating less green color. Retort samples got a deeper color than the other treatments, with a decrease in L*- value for both stem and floret. The origin, maturity, and blanching treatment (not known) of these samples must be considered and could result in individual differences. Different sizes of the pieces could also affect the color difference, especially for the thermally treated samples (MW and retort).

Table 4. The color difference (TCD)* for the stem (A) and floret (B) of cauliflower was measured after HPP (400 and 600 MPa), microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

A. Stem							
Storage (days)	**Control**	**HPP**				**Retort**	**Microwave**
	0.1 MPa	**400 MPa**		**600 MPa**		**96 °C**	**320 kJ**
	0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
0	-	2.9±1.7 $^{A, a}$	3.3±1.3 $^{A, ab}$	3.2±1.5 $^{A, ab}$	3.2±1.4 $^{A, ab}$	4.2±2.0 $^{A, b}$	3.4±1.6 $^{A, ab}$
14	nm**	2.8±1.2 $^{A, a}$	3.2±1.3 $^{A, ab}$	3.7±1.4 $^{A, abc}$	3.9±1.9 $^{A, cb}$	5.3±1.7 $^{BA, c}$	4.5±0.9 $^{B, cd}$
28	nm	3.5±1.7 $^{AB, a}$	3.7±1.1 $^{A, a}$	3.4±1.5 $^{A, a}$	3.7±1.5 $^{A, a}$	6.3±2.2 $^{B, b}$	nm
42	nm	4.2±2.2 $^{B, a}$	3.9±1.4 $^{A, a}$	3.4±1.6 $^{A, a}$	4.0±1.5 $^{A, a}$	9.0±2.2 $^{C, b}$	nm
B. Floret							
Storage (days)	**Control**	**HPP**				**Retort**	**Microwave**
	0.1 MPa	**400 MPa**		**600 MPa**		**96 °C**	**320 kJ**
	0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
0	-	4.0±2.4 $^{A, a}$	4.7±2.9 $^{A, ab}$	4.2±1.9 $^{A, a}$	4.2±2.3 $^{A, a}$	6.0±1.9 $^{A, b}$	5.4±2.6 $^{A, ab}$
14	nm	4.3±1.9 $^{A, a}$	5.1±2.1 $^{A, ab}$	4.2±2.0 $^{A, a}$	4.5±2.1 $^{AB, ab}$	7.8±2.6 $^{B, c}$	6.0±1.6 $^{A, b}$
28	nm	4.5±2.2 $^{A, a}$	4.7±2.3 $^{A, a}$	4.7±2.0 $^{A, a}$	5.7±2.5 $^{B, a}$	7.8±1.5 $^{B, b}$	nm
42	nm	4.8±2.5 $^{A, a}$	5.1±2.2 $^{A, a}$	4.6±2.1 $^{A, a}$	5.0±2.0 $^{AB, a}$	10.9±2.6 $^{C, b}$	nm

*Each color value (TCD) in the table is the mean ±standard deviation from processing parallels (n=3). The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and days (same row).

**Not measured.

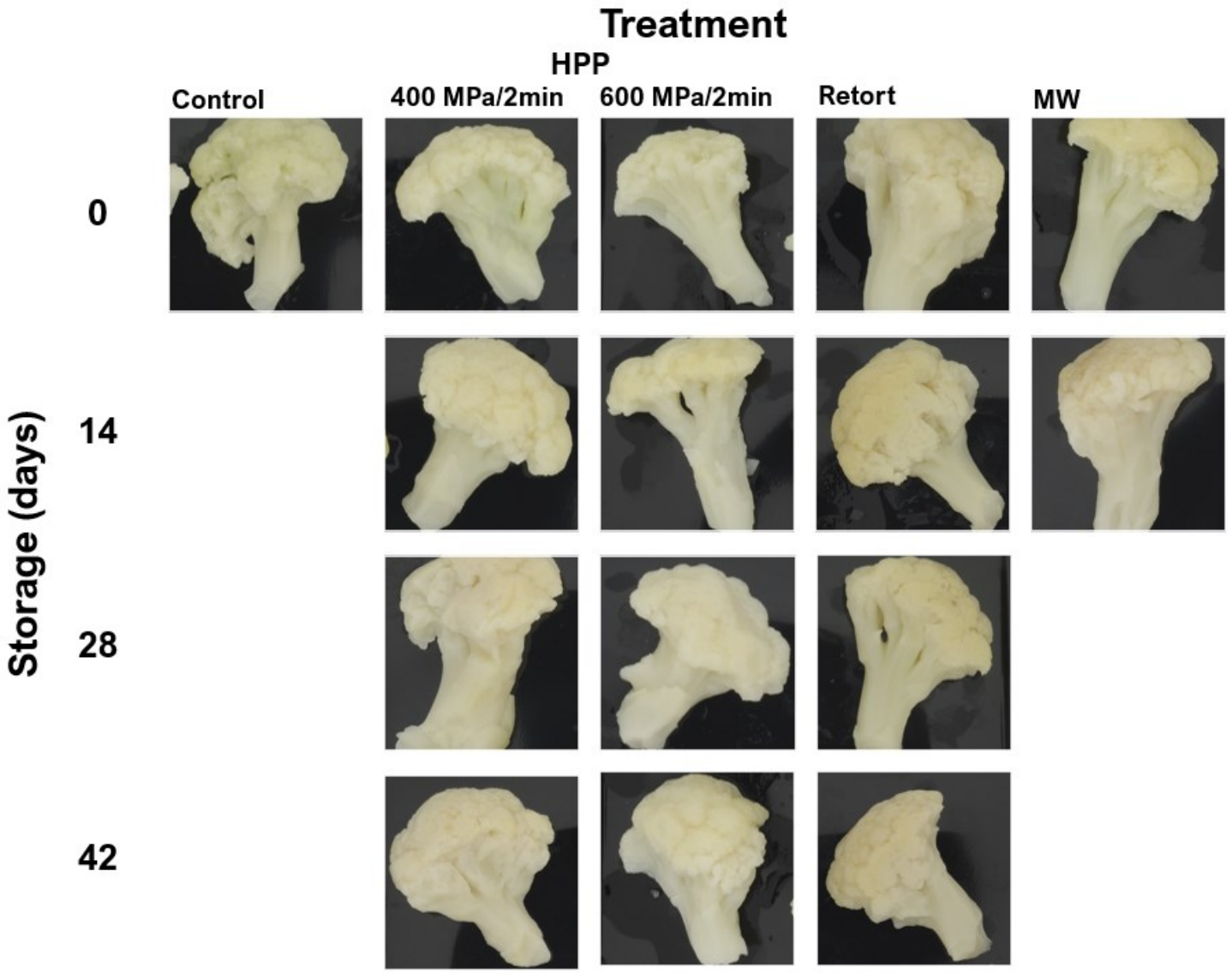

Figure 16. Visual comparison of selected pieces of cauliflower, HPP (only 400/600 MPa for 2 min) & retort samples until day 42, and MW samples until day 14.

For the heat treatment of vegetables, color degradation is closely related to enzymatic activity. Baardseth and Næsset (1989) studied heat inactivation of enzymes in cauliflower and found that active polyphenol oxidase (PPO) could give browning of the color. Rayan, Gab-Alla, Shatta, and El-Shamei (2011) found that lipoxygenase (LOX) was the most heat-stable enzyme during blanching of cauliflower (5 min, 50-100 °C). At 95 °C LOX had a 5.6 % residual activity, and at 100 °C the enzyme was completely inactivated. They further mention that LOX could result in loss of pigments; this could be the reason for the difference in color for MW at day 14, due to incomplete inactivation of this enzyme. Microbial activity can also lead to increased enzyme activity and could also explain the differences.

Enzymes can also be very pressure tolerant and maybe the reason for differences in TCD for the HPP samples during storage. The inactivation kinetics of enzymes during HPP is complex. It is generally believed that pressures above 300 MPa can induce irreversible denaturation of enzymes at room temperature, but some enzymes such as PPO in blueberries can increase their activity at 700 MPa at room temperature (Terefe et al., 2013). Palou et al. (2000) found that LOX in HPP guacamole had a residual activity of 41.2 % at 689 MPa (5 min), while PPO had a residual activity of 50.8 % after the same treatment. Arroyo et al. (1999) reported a slight browning of the color of cauliflower subjected for 350 MPa (5 °C, 30min), and explained this by peroxidase activity. They further measured the activity of peroxidase in tomato, lettuce, and onion. At 300-350 MPa the activity of this enzyme increased, and 400 MPa was not enough to inactivate it. They also discussed that some enzymes could be inactivated by heat while being reactivated during pressure treatments. Further investigation of how enzymes behave in cauliflower during altered HPP, and in combination with heat should be looked at. According to a review by Serment-Moreno et al. (2014), they found that limited experimental conditions made the generalization of HPP enzyme inactivation difficult, a case-by-case approach could therefore be needed.

Anese, Nicoli, Dall'aglio, and Lerici (1994) found that pressures up to 900 MPa were necessary to inactivate extracts of POD and PPO. More recently, Chakraborty, Rao, and Mishra (2015) found that for pineapple puree, HPP in combination with heat (600 MPa/70 C/20 min) gave 90 % inactivation of PPO. In general, pressures above 700 MPa are needed to inactivate PPO (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010), thus, to remain the fresh color and nutrition (polyphenols) of HPP cauliflower during storage, heat could be suggested as an additional step. At the same time, heat and pressure work antagonistically on each other in terms of the inactivation of enzymes. For instance, Terefe, Delon, and Versteeg (2017) found that high pressure inhibits the thermal inactivation of POD in blueberries. They also point out that the exact mechanisms of how pressure affects enzymes are not fully understood, and the antagonistic effect could vary between plants.

4.3 Texture

Changes in the texture of the cauliflower were measured by a shear force test. This type of test can indicate the resistance of tissue fracture in terms of the peak force during the test (Trejo Araya et al., 2007). As can be seen from Table 5, none of the HPP and MW treatments at day 0 resulted in a significant difference ($p<0.05$) from the control sample. The only exception from this was 600 MPa, 5 min at day 14 ($p>0.05$). During storage, the texture remained firm for both HPP and MW samples. Nevertheless, the SD was high in most of the samples, indicating some limitations for the method applied. This was expected since the thickness and size of the stem did vary. The reason for this was that frozen prepacked cauliflower was used, with varying sizes. Some did not have the stem intact, therefore some sorting had to be done. This could result in some biased and non-uniform distribution of the samples. An increase in the number of samples measured, and modifications of the method could be necessary. It could be a fact that some bags contained smaller pieces of stems, and therefore led to a lower force of texture. This could be the reason for the 600 MPa, 5 min (day 14) stands out. Another reason could be that this sample only had one run tested. The second run could not be used, because the results were 3 times higher than all the other samples, indicating possible errors with the settings of the texture analyzer.

Table 5. The firmness (force kg) * for the stem of cauliflower was measured before (control) and after HPP (400 and 600 MPa), microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

Storage (days)	**Control**	**HPP**				**Retort**	**Microwave**
	0.1 MPa	**400 MPa**		**600 MPa**		**96 °C**	**320 kJ**
	0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
0	15.9±3.8 ^{a}	14.4±3.8 $^{A, a}$	14.5±4.3 $^{A, a}$	15.6±4.5 $^{A, a}$	12.5±6.4$^{AB, a}$	0.8±0.3 $^{A, b}$	17.1±4.7 $^{A, a}$
14	nm **	11.3±5.3 $^{A, a}$	12.4±4.0 $^{A, a}$	13.6 ±3 $^{A, a}$	10.4±5.8 $^{B, a}$	0.5±0.1 $^{AB, b}$	13.5±3.8 $^{A, a}$
28	nm	16.6±3.9 $^{A, a}$	14.3±5.9 $^{A, a}$	13.1 ±5.3 $^{A, a}$	15.7±5 $^{A, a}$	0.5±0.1 $^{B, b}$	nm
42	nm	17.1±3.7 $^{A, a}$	17.3±2.6 $^{A, a}$	16.0 ±3.3 $^{A, a}$	12.6±3$^{AB, a}$	0.6±0.2 $^{AB, b}$	nm

*Each firmness value (force kg) in the table is the mean ± standard deviation from processing parallels (n = 3). The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and days (same row).

**Not measured.

Samples treated with a conventional retort showed significant differences from all the other treatments and the control ($p<0.05$). On day 0, the retort sample had a 95 % relative reduction in texture compared to the non-treated control, indicating a soft texture. With this little texture left in the cauliflower, it is unlikely that this would be a beneficial sensory attribute for the consumer. The process holding time of heating at 90-

96 °C for 45 min is likely to cause loss of turgor pressure, and degradation of the pectin in the cauliflower (Van Buggenhout et al., 2009). The chosen holding time was based on an industry-relevant condition, but other holding times should have been looked at. Vervoort et al. (2012) retorted carrots with a holding time of 19.6 min at 90 °C and reported this as a severe heating process.

The overall picture of the HPP samples shows that the texture remains intact both during storage and between the different conditions used. The time of the HPP did not result in a significant difference between the HPP samples ($p>0.05$). Consequently, from an industrial point of view, the use of 2 min instead of 5 min could be beneficial. On a general basis, HPP of vegetables has been found to yield low changes in pectin solubilization and depolymerization (Vervoort et al., 2012), but this is dependent on the type of vegetable. For instance, Arroyo et al. (1997) observed tomato loosened the skin but retained its firm texture during HPP from 300-400 MPa (10 °C, 20 min), whilst the flavor and color remained unchanged. Alvarez-Jubete et al. (2014) found that the texture of white cabbage significantly increased compared to the non-treated samples at 600 and 400 MPa at 20 °C. They suggested that HPP could liberate pectin methylesterase because of cell rupture. In turn, the de-esterified pectin can form a gel network that could give increased firmness in vegetables. This could also explain the HPP preservation of the texture of the cauliflower samples.

Clariana, Valverde, Wijngaard, Mullen, and Marcos (2011) observed a similar trend, where swedes subjected at 600 MPa (20 °C, 5min) yielded significantly higher texture than 400 MPa during compression. They explained this with a possible inactivation of polygalacturonase (PG) at pressures above 500 MPa. When the same authors measured the cutting force, 600 MPa of swede resulted in a similar texture as the control, while 400 MPa increased the texture further. For carrots, Trejo Araya et al. (2009) found slightly higher cutting forces of the HPP (600 MPa, 2min) until day 14 of cold storage, compared to non-treated carrots. This trend was also observed for the HPP cauliflower during day 42 of storage. More recently, Hu et al. (2020) subjected frozen pumpkin (-20 °C) to pressures (100-600 MPa/2 min) and found a significant reduction in the texture attribute «hardness». At 400 and 600 MPa, they reported a decrease in hardness of about 42 % compared to the control. During storage of 7 days, the authors observed a reduction in hardness of 54 % (600 MPa) and 36 % (400 MPa). These findings were not in agreement with the cauliflower samples and could be a result of different plant structures.

The high firmness of cauliflower during HPP could be explained by the findings of Préstamo and Arroyo (1998). They found that tougher plant tissue such as cauliflower preserved texture better than spinach leaves processed at 400 MPa for 5 min and 30 min. They further noticed that HPP of cauliflower (400 MPa/30 min/5 °C) resulted in cavity formation and a firm and soaked texture and appearance. They explained this by loss of turgor pressure within the cell and the presence of fluid in intercellular spaces

that were previously filled with gas. The loss of fluid could contain water and other components such as salt, vitamins, and other nutrients. This fluid could also induce chemical reactions, i.e., with the release of enzymes. Further, they observed that the cauliflower was near the control (non-treated) with an acceptable firmness and flavor. Arroyo et al. (1999) conducted a sensory evaluation of HPP cauliflower (400 and 350 MPa /5 °C/30 min) and they reported the texture as «firm».

Firmness remained high for the MW samples during storage. Several other authors also found high retention of texture during MW of different vegetables. Zhong et al. (2017) found that the texture of frozen broccoli during MW was dependent on the size of the steam bag. Bags with a small surface area had a softer texture because this generated steam faster. They explained that higher water loss of steam gave a less firm vegetable. For this research, the packages did not have a valve to eject the steam. The importance of the size of the package during MW should be investigated further.

Bongoni (2014) tested the effect of steaming and boiling methods on the sensory texture of broccoli. Medium-firm broccoli was preferred in terms of texture, whereas high firmness got a lower sensory score. Since this cauliflower is intended to be a part of an RTE dish, further heating would be necessary. This could also influence the texture further and should be investigated. Therefore, the high firmness after HPP and MW could be positive.

4.4 Drip loss

Drip loss of control, blanched, and cooked cauliflower is presented in Table 6. The lowest drip loss ($p<0.05$) was observed for MW cauliflower. The drip loss was significantly higher for high pressure treated cauliflower than it was for untreated samples (control). There was not a significant difference ($p>0.05$) for drip loss at treatment time for 2 and 5 min within each pressure level. For each treatment, the drip loss remained stable during storage. On the other hand, for some days the SD was high, for instance with day 28 (400 MPa, 2min). A higher number of replicates could have resulted in less variability.

The use of a high vacuum (99.5%) and pressurization might explain some of the high drip loss of HPP cauliflower. Trejo Araya et al. (2007) HPP carrots (100-550 MPa, 2/10/30 min), reported that pressures cause instantaneous deformation of plant cells that gives turgor loss and loss of water. The findings from Préstamo and Arroyo (1998), described above in section 4.3, could also describe the drip loss of HPP cauliflower. Clariana et al. (2011) analyzed moisture loss during compression, they observed higher water losses in HPP swedes at 400 than 600 MPa. The water loss during cutting when measuring the texture of cauliflower could be interesting to investigate further.

Table 6. The drip loss (%) * for pieces of cauliflower measured before (control) and after HPP (400 and 600 MPa), microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

Storage (days)	Control	HPP				Retort	Microwave***
	0.1 MPa	400 MPa		600 MPa		96 °C	320 kJ
	0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
0	18.0±4.8 $^{A, bc}$	31.7±7.6 $^{A, a}$	32.3±6.0 $^{A, a}$	27.2±5.6 $^{A, ab}$	33.0±7.4 $^{A, a}$	22.8±7.4 $^{AB, ab}$	5.2±0.3 $^{A, c}$
7	17.9±3.9 $^{A, b}$	32.9±9.4 $^{A, a}$	28.5±3.3 $^{A, a}$	30.6±6.2 $^{A, a}$	29.9±5.1 $^{A, a}$	16.6±2.5 $^{B, b}$	9.7±1.4 $^{B, b}$
14	nm**	27.4±6.1 $^{A, ab}$	29.5±5.8 $^{A, ab}$	34.3±6.6 $^{A, a}$	26.6±4.0 $^{A, ab}$	21.2±8.5 $^{B, b}$	8.0±2.4 $^{AB, c}$
28	nm	32.3±12.7 $^{A, a}$	27.4±8.2 $^{A, a}$	29.9±6.0 $^{A, a}$	27.0±8.3 $^{A, a}$	22.3±5.0 $^{B, a}$	nm
35	nm	nm	nm	30.7±5.0 $^{A, ab}$	31.6±5.6 $^{A, a}$	23.9±5.1 $^{AB, a}$	nm
42	nm	nm	nm	31.5±5.7 $^{A, a}$	31.5±5.3 $^{A, a}$	32.7±7.4 $^{A, a}$	nm

*Each drip loss value (%) in the table is the mean ± standard deviation from processing parallels (n=3). The capital letters (A, B, and C) indicate a significant difference ($p< 0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ($p< 0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and days (same row).

**Not measured.

***Drip loss was weighed from a bigger package than a single piece as the other treatments.

For the retorted samples, they were «grainy» and overcooked, resulting in small pieces left in the bag when weighing them. This is likely to give inaccuracies for the results here. When measuring the weight of cauliflower, it was observed that smaller pieces lost more water than the bigger pieces that remained more intact. When weighing in pieces, a weight of 5-10 g was targeted, but some bags contained several smaller pieces, whilst others contained one larger piece.

The use of frozen cauliflower could also explain high drip loss, since freezing damages the plant cell structure by the expansion of the intracellular fluid (Xin et al., 2015). Results for the MW samples were obtained differently than the others, this makes a comparison between the other treatments difficult. As described in section 4.1, this was because single-packed pieces were not suitable for the MW treatment. Nevertheless, low drip loss during MW treatment has also been reported by Wang et al. (2017), who looked at red bell peppers. They observed that the drip loss increased with the increased power of the MW (0.65/0.75/0.90 kW, 100 s), 1.7, 2.4, and 4.1 %, respectively. Compared to hot water blanching, MW significantly reduced the drip loss. Ruiz-Ojeda & Peñas (2013) also observed the same tendency in MW of green beans. As a result, these finding could indicate that the observed drip loss of cauliflower during MW are reasonable.

4.5 Dry matter content (DM)

The dry matter (DM) content of food consists of all its constituents excluding water. It can vary between and within different cultivars of plants (Volden et al., 2009), and is useful when presenting chemical data as an amount per dry weight (DW). Table Table 7 represents the influence of different processing conditions on the percentage of dry

matter content in pieces of cauliflower. The DM content was also measured for the different samples used for chemical analysis (numbers not shown here). This was because residual water after freeze-drying needed to be removed to obtain the dry weight for nutrient contents.

The dry matter content did not vary significantly within each treatment during storage, and few significant differences between treatments were observed. On day 0, the HPP samples represent the mean of only one experiment instead of two experiments from the other days. The high SD for retort and MW day 0 resulted in no significant differences between the other treatments. The general trend was that the DM content remained stable during storage for each treatment.

Table 7. The dry matter content (%) * for pieces of cauliflower measured before (control) and after HPP (400 and 600 MPa). microwave. and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

Storage (days)	Control	HPP				Retort	Microwave
	0.1 MPa	400 MPa		600 MPa		96 °C	320 kJ
	0 min	2 min	5 min	2 min	5 min	45 min	10 min
0	6.9±0.3 ^{a}	6.1±0.7 $^{A, a}$	6.6±0.2 $^{A, a}$	6.3±0.6 $^{A, a}$	5.8±0.1 $^{A, a}$	7.3±1.2 $^{A, a}$	6.8±0.7 $^{A, a}$
14	nm**	6.3±0.4 $^{A, b}$	6.8±0.4 $^{A, ab}$	6.3±0.3 $^{A, b}$	6.2±0.3 $^{A, b}$	7.3±0.8 $^{A, a}$	6.5±0.3 $^{A, ab}$
28	nm	6.5±1.0 $^{A, a}$	6.5±0.8 $^{A, a}$	6.2±0.3 $^{A, a}$	6.4±0.2 $^{A, a}$	7.0±0.3 $^{A, a}$	nm
42	nm	6.7±0.4 $^{A, ab}$	6.6±0.5 $^{A, ab}$	6.3±0.1 $^{A, ab}$	6.1±0.4 $^{A, b}$	7.2±0.4 $^{A, a}$	nm

*Each DM value (%) in the table is the mean ± standard deviation from processing parallels (n=3). The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and days (same row).

**Not measured.

The DM content of raw cauliflower seems to vary between different studies. Florkiewicz, Socha, Filipiak-Florkiewicz, and Topolska (2019) reported 8.42 % DM of raw cauliflower, while Cebula, Kunicki, and Kalisz (2006) reported 6.57 % DM. Dos Reis et al. (2015) reported a 4 % reduction in DM of MW cauliflower (0.8 kW, 4min), compared to the control (9.7 % DM). Ahmed and Ali (2013) reported a significantly lower DM content in MW cauliflower (8.87 %) compared to the control (11.36 %). They also observed a higher DM content in boiled cauliflower since the plant cells gelatinize and take up water during heating. A similar trend could not be found for the present study, but the retorted samples showed a slightly higher DM content, possibly due to leakage of water during heating. A possible source of error could result from the evaporation of the cauliflower samples during preparation and weighing. This could be possible

since the same pieces that were used for DM were first used to measure color, followed by texture analysis.

Other reasons for variation could be that frozen pre-blanched cauliflower was used. Kapusta-Duch et al. (2019) reported a DM of 9.18 % of raw purple cauliflower. During frozen storage, they observed a significant increase in DM to 11.7 %. Similarly, Gębczyński and Kmiecik (2007) reported 7.28 % DM in raw cauliflower and 6.61 % DM for blanched cauliflower. When this blanched sample was further frozen at -30 °C, a 7.56 % DM was observed. For HPP samples, Melse-Boonstra et al. (2002) reported 7.7 %, and 6.8 % DM, for control and HPP (200 MPa, 5min) cauliflower, respectively. No other studies have been found for DM in HPP cauliflower. For carrots, Vervoort et al. (2012) reported significant reductions in DM % during HPP (600 MPa, 10 °C, 10min) compared to the control. Interestingly, the same HPP insignificantly changed the DM content compared to both mild (70 °C, 7.5 min) and severe (90 °C, 19.6 min) thermal pasteurization of carrots.

4.6 Chemical analysis

Only the HPP cauliflower treated at 600 MPa for 2 min were chosen to be further analyzed chemically (until day 28). For day 0 samples for the retort, MW and control were analyzed for comparison. The choice of 600 MPa, 2 min was selected due to lower bacterial counts (compared to the other HPP parameters), and because 5 min did not differ significantly from 2 min of the above-mentioned quality parameters. Another reason being that the freeze-dryer at Nofima was not functioning, and this led to delays by drying the samples at NTNU instead.

4.6.1 Antioxidant activity (DPPH)

Studies performed on antioxidant activity (AOC) are scarce and sometimes contradictory. This could reflect the different methods used to quantify AOC in different foods (Escobedo-Avellaneda et al., 2011). In sum, many compounds absorb the same wavelength of the DPPH radical, therefore they may interfere with the analysis (da Silva Oliveira et al., 2019). Results in Table 8 represent the influence of different processing conditions on the AOC (%) in pieces of cauliflower. The results reveal that the non-treated control had the highest AOC content ($p<0.05$), followed by MW, HPP, and retort at day 0. During storage of the HPP samples, a significant increase ($p<0.05$) was observed on day 28. The loss of AOC during retorting is well known and could be due to leaching or accelerated degradation of antioxidants (Roy, Takenaka, Isobe, & Tsushida, 2007).

No studies on the effects of HPP on AOC in cauliflower have been found. For other vegetables, the effects of HPP on AOC have been shown to vary (Escobedo-Avellaneda et al., 2011). Alvarez-Jubete et al. (2014) subjected white cabbage for HPP (200/400/600 MPa, 20/40 °C), and found that 600 MPa gave significantly higher retention of AOC with almost 4 times higher activity compared to 200 and 400 MPa. Clariana

et al. (2011) also found the same trend, where swedes pressurized at 600 MPa were insignificant higher than the control and had 2.6 times higher AOC than at 400 MPa. This could indicate that lower pressure levels would not be beneficial for cauliflower as well, although this would have to be tested first. The authors also found a positive correlation of AOC with both AA and TPC.

Table 8. The DPPH antioxidant activity (%) * of cauliflower was measured before (control), and after HPP, retort, and microwave treatment. Only samples of HPP were measured until day 28 of storage.

Storage (days)	Control	HPP	Retort	Microwave
	0.1 MPa	600 MPa	96 °C	320 kJ
	0 min	*2 min*	*45 min*	*10 min*
0	72.1 ± 2.5^{A}	$52.4\pm6.9^{C, b}$	49.8 ± 2.3^{C}	62.1 ± 2.8^{B}
14	nm**	46.5 ± 4.6^{c}	nm	nm
28	nm	68.3 ± 4.3^{a}	nm	nm

*Each AOC value in the table is the mean ± standard deviation of three experiments. The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and day (same row). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column).

**Not measured.

Venzke Klug et al. (2020) suggested that heating with MW could break down cell walls leading to enhanced extractability. They found a higher percentage of AOC in MW cowpea puree (8kW, 35 s) than in the control sample. Ahmed and Ali (2013) found that MW (1kW, 3.5 min) of cauliflower had high retention of AOC (58.24%), which was a 15 % decrease from the control. They explained the retention with the fact that MW retained a high amount of AA and TPC also. Their results show resemblance to the present study also, where a 14 % reduction of AOC in MW samples compared to control. In addition, Dos Reis et al. (2015) observed higher AOC in broccoli and cauliflower during MW (0.8 kW, 4min). Pellegrini et al. (2010) reported no change in the AOC for MW (0.3 kW, 20 min) cauliflower compared to frozen control. On the contrary, Wachtel-Galor, Wong, and Benzie (2008) reported low AOC and TPC for MW cauliflower (0.75 kW, 5/10min). The authors MW cooked 5 g of cauliflower in 100 ml of water, and they explained this with great loss of nutrients in the water.

4.6.2 Total phenolic content (TPC)

Phenolics can influence the taste, flavor, and appearance of food, and due to their nutritive properties, they can be considered functional compounds (Barrett & Lloyd, 2012). Results in Table 9 represent the influence of different processing conditions on the content of total phenolic content (mg/100g dry weight (DW)) in pieces of cauliflower. The results show a similar variation among the different samples as for the antioxidative capacity results. The differences throughout storage of HPP remained stable over 28 days, with a 12 % reduction at day 28 compared to the control.

Concerning the MW samples, these were not significantly different from the control ($p>0.05$). This could be a result of the short temperature and processing time, yielding less drip loss, and leaching of the cells. The retorted samples had the least retention of total phenolics compared to the other ($p>0.05$), which is likely the result of heat degradation and leaking of cells (Rickman et al., 2007). The enzyme PPO can reduce the content of phenolics, and residual activity of this could explain the lower TPC of the HPP cauliflower. Palou et al. (2000) found a 50.8% residual activity of PPO in guacamole during HPP (689 MPa/5min), this indicates that it could be possible that PPO remained active in the cauliflower and resulted in a reduction of the TPC. Further description of the inactivation of this enzyme was described in section 4.2.

Table 9. The total phenolic content expressed as mg gallic acid equivalents (GAE)/100 g DW * of cauliflower was measured before (control), and after HPP, retort, and microwave treatment. Only samples of HPP were measured until day 28 of storage.

Storage (days)	**Control**	**HPP**	**Retort**	**Microwave**
	0.1 MPa	**600 MPa**	**96 °C**	**320 kJ**
	0 min	*2 min*	*45 min*	*10 min*
0	579.6±19.4^{A}	506.6±55.6$^{B, a}$	422.2±40.3^{C}	592.0±36.5^{A}
14	nm**	477.8±47.9^{a}	nm	nm
28	nm	510.8±24.6^{a}	nm	nm

*Each TPC value in the table is the mean ± standard deviation of three experiments. The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and day (same row). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column).

**Not measured.

Other studies on HPP effects on TPC in vegetables show that in most cases, pressure increases the concentration of phenolic compounds. For instance, Alvarez-Jubete et al. (2014) found that among HPP white cabbage, 600 MPa yielded the highest retention of total phenols (393 mg GAE/100 g DW) compared to lower pressures. Clariana et al. (2011) also observed a similar trend in swedes, where 600 MPa gave the highest retention of TPC (399 mg GAE/100 g DW) compared to lower pressures. These findings indicate that higher pressures for cauliflower could be beneficial in nutrient retention. Venzke Klug et al. (2020) MW (8kW, 35 s) and pressurized cowpea puree (550 MPa, 10 min, 23 °C) and found that the samples retained about 91-94 % of their initial TPC during 21 days of storage at 5 °C.

Florkiewicz et al. (2019) tested the effects of steaming, boiling, and sous-vide cooking of cauliflower. For their sous-vide samples, this increased the availability of total phenolic contents by 18 % (mg GAE/ kg fresh weight), compared to the control. They explained that an increase could be possible when new compounds with antioxidants are formed, such as through Maillard reactions. This could bear some resemblance to the present work. The authors also found that for 5 days of storage Romanesco cauliflower had a higher content of phenolic acids compared to the control. Also, for MW samples,

there has been suggested that this treatment could enhance the extractability of antioxidants (Swamy & Muthukumarappan, 2021), this could be due to disruption of polyphenol-protein complexes (Volden et al., 2009). Ahmed and Ali (2013) found that MW of cauliflower (1 kW, 3.5 min) yielded an 18 % reduction (639 mg GAE/ 100g DW) in TPC compared to the control. Nevertheless, the MW treated their cauliflower in a bowl with 10 ml water, with 200 g of cauliflower, thus some evaporation and loss of nutrients would be expected. For the present study, the cauliflower was packed vacuum-sealed, possibly leading to higher retention of TPC and other nutrients. Dos Reis et al. (2015) also reported a slight increase in TPC for MW (0.8 kW, 4min) treated cauliflower (2.4 %) and broccoli (14.4 %) compared to the control. Further, Pellegrini et al. (2010) found no changes in polyphenol content during MW (0.3 kW, 20 min) cauliflower compared to frozen control.

For the HPP samples on days 14 and 28, an acidic smell was observed, indicating that LAB bacteria could have produced organic acids such as lactate. As was described in section 2.9.3, organic acids can interfere with the Folin reagent and give an appearance of a higher level of polyphenols than what it is. Measuring the pH during storage would be necessary. The content of ascorbic acid in the samples could also interfere with the TPC because it can be oxidized by the Folin reagent resulting in reduced (colored) forms that appear as phenolics (Zhang & Hamauzu, 2004).

4.6.3 Ascorbic acid (AA)

Ascorbic acid (AA) is regarded as one of the most heat-sensitive vitamins and is therefore used as a key indicator of the effects of processing on vitamins (Davey et al., 2000). Results in Table 10 represent the influence of different processing conditions on the content of AA (mg/100g dry weight (DW)) in pieces of cauliflower. The results reveal that MW contained the highest levels of AA (934.7 mg/100g DW), followed by control, HPP, and retort with the lowest ($p<0.05$) content of AA (530.6 mg/100g DW). The retorted samples resulted in a 40 % reduction in AA compared to the control. This indicates that heating for long periods thermally degrades AA. Murcia, López-Ayerra, Martinez-Tomé, Vera, and García-Carmona (2000) reported an 84 % reduction in AA for retorted broccoli (121 °C, 30 min).

When seeing the AA content concerning the drip loss (section 4.4), both HPP and retort shows a high drip loss, whereas MW had a significantly lower drip loss than the others ($p<0.05$). This could be one of the reasons for the high AA content for MW cauliflower. Also, the mild heating of MW (described in section 4.1) could result in low losses of AA. During storage of HPP samples, the content of AA remained high (above 730 mg/100g DW). HPP is a mild processing condition that does not affect low molecular weight molecules such as AA much, because of the low compressibility of covalent bonds (Heinz & Buckow, 2009). On the other hand, some degradation of AA could result due to remaining active enzymes that were not inactivated by the 600 MPa pressure.

Table 10. The content of ascorbic acid (mg/100g DW) * of cauliflower was measured before (control), and after HPP, retort, and microwave treatment. Only samples of HPP were measured until day 28 of storage.

Storage (days)	Control	HPP	Retort	Microwave
	0.1 MPa	600 MPa	96 °C	320 kJ
	0 min	*2 min*	*45 min*	*10 min*
0	890.9±53.6^{AB}	862.1±45.6$^{B, a}$	530.6±7.0^{C}	934.7±29.2^{A}
14	nm**	730.7±22.2^{b}	nm	nm
28	nm	790.4±95.0^{ab}	nm	nm

*Each AA value in the table is the mean ± standard deviation of one experiment. The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and day (same row). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column).

**Not measured.

The effects of HPP on AA in vegetables and fruits have been shown to vary. Sancho et al. (1999) mention that HPP might enhance the extraction of AA and found low changes in AA after pressurization (400/600 MPa, 30min) of strawberry coulis during storage over 30 days. In some studies, HPP (100/200 MPa, 10/20 °C) of green bell peppers showed a decrease of about 15–20% of AA, while red peppers showed an increase of about 10–20% (Castro et al., 2008) and yellow peppers an increase of 11 to 48%, all compared to blanching (Castro et al., 2011). Further, Alvarez-Jubete et al. (2014) found a significant reduction of AA in white cabbage after HPP (600/400 MPa, 20 °C, 5 min) compared to non-treated. Their control had about 840 mg AA/100 g DW, while 400 MPa gave under 100 mg/100 g DW, and 600 MPa yielded about 620 mg/100 g DW. A similar trend was also observed from Clariana et al. (2011), who subjected swede for the same HPP conditions as the mentioned study above, with 81 % retention of AA at 600 MPa (664 mg/100 g DW), while 400 MPa gave the lowest retention of AA (257 mg/100 g DW). They hypothesized that high retention at 600 MPa could be a result of higher inactivation of the POD enzyme.

Effects of MW on the AA content of vegetables have been studied by several authors. Among them, Pellegrini et al. (2010) reported high AA contents of raw frozen cauliflower (749 mg/100 g DW). When subjected to MW (0.3 kW, 20 min) a reduction to 473 mg/100 g DW was found. Similarly, Vallejo, Tomás-Barberán, and García-Viguera (2002) reported 38 % loss of AA during MW (1 kW, 5 min) of cauliflower. This is not in agreement with the present study, which showed a slight increase from the control. Further, research conducted by Ahmed and Ali (2013) found that MW (1 kW, 3.5 min) cauliflower led to high retention of AA, compared to boiling, steaming, and stir-frying. For the present study, since the cauliflower was processed in vacuum packages, this could lead to more retention of AA.

The high content of AA in the MW samples could also result from increased extraction and bioactivity of nutrients, as mentioned for AOC and TPC. Other reasons for the high retention could relate to the work conducted by Zhong et al. (2015). They found that

steamable bag microwaving of broccoli was a better method for retaining nutrients compared to traditional cooking methods. Moreover, they also studied the effect of the size of the steamable bag, squared versus rectangular shaped, and found that a more squared size gave better nutrient retention (690 mg/100 g DW) and better quality in terms of texture (Zhong et al., 2017). Although they looked at broccoli, this vegetable has a somewhat similar structure as cauliflower, and their results could be used for comparison. The authors had a valve in the package where steam was released, and they explained that AA could be lost with the water vapor. In this present study (my research), there was not a valve in the package, and this is likely to explain the higher retention of AA here (934 mg/100 g DW).

Some authors (Mazurek & Jamroz, 2015; Zhu et al., 2020) claim that a total ascorbic acid (TAA) value (defined as the sum of AA and DHAA) should be measured to give an accurate evaluation of the nutritional quality. This is because the oxidized form of AA, called DHAA, also has a biological activity, and can be reduced back to AA in the body. AA can be reduced to DHAA during the processing and storage of food through several conditions, such as the presence of high pH, light, high temperature, and oxygen (Zhu et al., 2020). On a general basis, it could be feasible that the cauliflower sampled in this study may be poor-quality raw material due to crop variation or prolonged storage, which could have resulted in extensive oxidation of AA to DHAA, even before processing, and therefore the lower concentration of AA.

4.6.4 Overall discussion of chemical analysis

For comparison of results with other studies on the antioxidant activity of *Brassica* vegetables, different extraction procedures and several methods have been used. Consequently, a comparison of results is difficult. Therefore, several studies highlight the need for standardization of the different methods being used (Ahmed & Ali, 2013; Podsędek et al., 2006) According to Prior et al. (2005), they point out that multiple assays need to be used to indicate the overall antioxidant capacity of vegetables and fruits. The authors also explain that antioxidants will vary in (1) solubility and phase of localization, (2) redox potential, and (3) specificity and mechanism of action, and therefore no single assay could provide all information about this.

Other issues of comparison with others are the following. Several studies report their results in mg/100g of fresh weight (FW), whereas it is recommended to report them in dry weight (DW) for better comparison since the water content will vary in vegetables (Galgano, Favati, Caruso, Pietrafesa, & Natella, 2007). Rickman et al. (2007) even claim that reporting nutrient results per FW could be misleading. Further, the retention of nutrients in vegetables will vary depending on the cultivar, production location, maturity stage, season, and harvesting, -storage, and processing conditions (Escobedo-Avellaneda et al., 2011).

Unfortunately, not all the samples that were being freeze-dried (FD) got the same treatment. Due to technical errors, samples for HPP day 0 and retort had to be taken out

during FD and back to the freezer, followed by a new process. For HPP samples on day 14, the process was paused to check the water content, and then frozen and re-dried due to high residual water. This indicates that the values obtained for chemical analysis for these samples might be higher than what was measured. A possible melting of the samples could result in quality defects and loss of nutrients (Alves-Filho & Roos, 2006). As such, the only FD processes that were not interrupted were the batch with MW at day 0, control samples, and HPP day 28 samples.

For further notice, consistent FD is essential for the comparison of results. Alves-Filho and Roos (2006) showed that the appropriate conditions of the FD process have been identified by many researchers using a method of trial and error. They explain that food materials differ in terms of structure, initial moisture content, and composition. Therefore, it is difficult to predict the behavior of the food during FD. This could also be the case for cauliflower, where more pre-trials should have been conducted before drying the main samples.

4.7 Overall assessment of the quality

A shelf-life extension of more than 35 days was the target for all treatments. HPP alone could not achieve this, due to high bacterial counts at days 35 and 42. Nevertheless, the texture and color were close to the control, indicating a fresh appearance during the first days of storage. The HPP at 600 MPa for 2 min also retained the nutrient quality, close to control samples. On the contrary, retorted samples remained under the detection limit (2.3 log cfu/g) until day 42, resulting in a possible extension of the shelf-life. Concerning other quality parameters, especially regarding texture, color, and nutrition, retorted cauliflower could be rejected by the consumer. Barbosa-Cánovas and Juliano (2008) mention that several retorted food products get a non-acceptable and low-quality value due to the long exposure of heat.

Relative to all other treatments, MW samples showed high nutrient retention at day 0 for all chemical analyses, except for DPPH. The raw frozen cauliflower had been blanched before freezing (conditions not known), thus some degrading enzymes could be inactivated here, whilst others could remain active, resulting in some degradation of nutrients. The MW received a mild, uneven heat treatment, and possibly resulted in some inactivation of enzymes. MW sample also had less drip loss than the other treatments, this could also explain the retention. On the contrary, if the MW samples were to be checked later during storage on day 14, it could be possible that the retention of nutrients had been lower. Especially when seeing this in the context of high bacterial counts at day 14, indicating that enzymes present here could also degrade nutrients. On the other hand, HPP (600 MPa, 2min) yielded high retention of nutrients during storage, especially in terms of AA and TPC content. Retort samples yielded low retention of AA and TPC, whereas AOC was not significantly different to HPP at day 0.

Industrial implementation of these new technologies (both HPP and MW) relies on cost-effective production, the complexity of operations, and added value to the final products (Rosnes et al., 2011). Moreover, Vervoort et al. (2012) claim that results obtained from lab-scale equipment could often result in conditions deviating from those in industrial-scale equipment. For MW treatment, several factors will affect the dielectric properties of the foods during heating, such as mass, shape, geometry, water content, chemical composition, position, the orientation of product, temperature, and frequency. A better understanding of all these is needed before further developments of this technology. Today there exist several solutions for packaging to achieve rapid and safe heating of the MW food. As an example, a company called Micwac offers a solution for MW pasteurization of RTE meals (Tang et al., 2018). They have their patented solution where steam is generated in the food package, and at a certain point, the steam will come out through a valve. When the steam is ejected, a vacuum will occur, and the valve automatically seals. This can give a better distribution of the heat, and no further processing is needed afterward. The company claim that a shelf-life of over 30 days at 8 °C can be achieved with their solution (Tang et al., 2018). For HPP, spores still represent a challenge for low-acid foods. Therefore, additional synergistic additives and processing steps are needed. The retorted samples confirmed that high heat for long times degrades the food quality, although low bacterial counts were found.

As a final remark, it is important to highlight that this work is a part of something wider. Although cauliflower was chosen for this experiment, the RTE dish from the industrial partner consists of several other vegetables, sauce, preservatives, and spices. The different food components will likely affect how the different processes applied here will change the food quality. For MW and retort treatment, it is expected that the heating pattern will be different in this multi-component food. However, since HPP works independent of the size and geometry of the food, less change could be expected here. The addition of different salts can also improve the texture of food during processing, and lowered pH can increase the shelf-life. Thus, there are several potentials for how these technologies can affect this RTE dish.

5. Conclusion

The impact of HPP, MW, and retorting on the quality of cauliflower have been investigated. During storage, pressures at 600 MPa for 2 min resulted in minor changes in the texture, color, drip loss, dry matter, and nutritional value compared to the control. Regarding bacterial counts, 600 MPa resulted in better preservation compared to 400 MPa, with moderate bacterial counts until day 28 of storage. After day 28 to day 42, high bacterial counts were observed (up to 7.5 log cfu/g) for 600 MPa. Low bacterial counts of both aerobic and anaerobic spores were detected for all treatments. Since the holding time of pressure treatment did not significantly impact the physical quality, a processing time of 2 min can be advised. To obtain better preservation and quality, a combination of HPP with other treatments such as heat should be investigated further. The retorted samples had a shelf-life of up to 42 days, but the color and texture analysis showed significant reductions in TCD and firmness compared to the other treatments. This indicates that this treatment would not be preferable for consumption. MW offers several opportunities as a technology, but the selected process parameters in this study resulted in a short shelf-life, thus optimization of the MW treatment is needed. Nevertheless, MW cauliflower at day 0 showed minor changes in quality in terms of color, texture, drip loss, dry matter, and nutritional value, but from an industrial perspective, an increase in shelf-life is needed. In conclusion, both HPP and MW offer potentials for enhancing the quality of cauliflower, but further investigations are needed.

6. Further work

This thesis studied the processing of cauliflower separately. Further work needs to address the composition and quality of cauliflower when it is mixed with other ingredients (such as a ready-to-eat dish). Also, sensory analysis of the taste, texture, and color of the processed cauliflower should be carried out to ensure that this product is both appealing to the senses and safe. In addition, it would be interesting to investigate the processed cauliflower when reheated, as it would in an RTE meal. The microbial flora also needs to be further investigated, where different target pathogens could be inoculated in the cauliflower. Since the nutritional analysis was only performed for the 600 MPa-2 min samples, it would have been of interest to do a nutritional analysis to evaluate and compare with other pressure and time combinations. In addition, an investigation of how supplementary processes and additives affect both HPP and MW would be of interest since these can have a potential synergistic effect on the shelf life and quality.

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Appendix

Appendix A. Temperature data for retort treatment

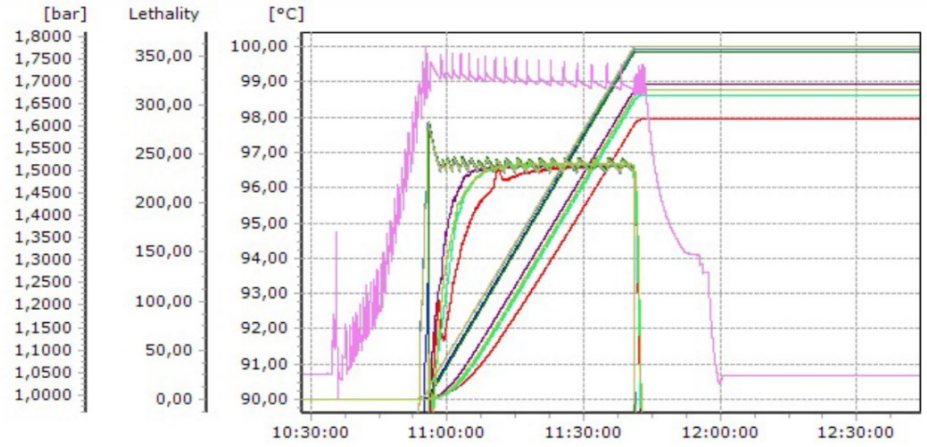
Retort treatment 1

A.

Operator:	Master	Time Zone:	(UTC+01:00) W. Europe Standard Time
Process:	96 °C 45 min	Vessel:	Autoklav statisk
Session Start:	21.01.2021 10:28:09	Product:	Blomkål i krympeskål
Session Stop:	21.01.2021 12:44:16		
Session Name:	Blomkål CF		
Session Text:	N/A		

Validation Solutions

Graph



	Sensor ID	Name	Validation Equipment	Description	Sample Rate 1/2 (hh:mm:ss)	Type	Sensor group
	31517-1	Probe 1	13553	N/A	00:00:01/-	TC	N/A
	29935-2	Probe 2	13553	N/A	00:00:01/-	TC	N/A
	29932-3	Probe 3	13553	N/A	00:00:01/-	TC	N/A
	31196-4	Probe 4	13553	N/A	00:00:01/-	TC	N/A
	32790-5	Probe 5	13553	N/A	00:00:01/-	TC	N/A
	31513-6	Probe 6	13553	N/A	00:00:01/-	TC	N/A
	30064-7	Probe 7	13553	N/A	00:00:01/-	TC	N/A
	29937-8	Probe 8	13553	N/A	00:00:01/-	TC	N/A
	180681-64	Probe 9	13553	N/A	00:00:01/-	P	N/A

B.

Lethality Overview

Input parameter

Start Date :	21.01.2021
Ref. Temperature:	90,00 °C
Min. Temperature :	60,00 °C
Z-Value:	7,5 °C
D-Value:	1

Sensor lethality overview

	Name	Description	Start Time	End Time	Max. Value
	Probe 1 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	285,32
	Probe 2 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	353,79
	Probe 3 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	353,43
	Probe 4 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	321,41
	Probe 5 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	356,08
	Probe 6 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	309,81
	Probe 7 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	359,29
	Probe 8 (F0) Lethality 1	L-Calculation	10:28:09	12:44:16	315,86

Figure 1. (A.) Shows the heating distribution, were probe 1, 2, 3, 4, 6, and 8 were inserted in the cauliflower. Probe 9 is showing the pressure. Probe 5 and 7 is showing the temperature of the water. When calculating the F-value for the average temperature of all the probes inserted in the cauliflower, a value of F=193 min was found. Also, the average temperature remained at 90-96-90 °C for 45.76 min. (B.) Shows the F-value for each probe (also called L-value as indicated here).

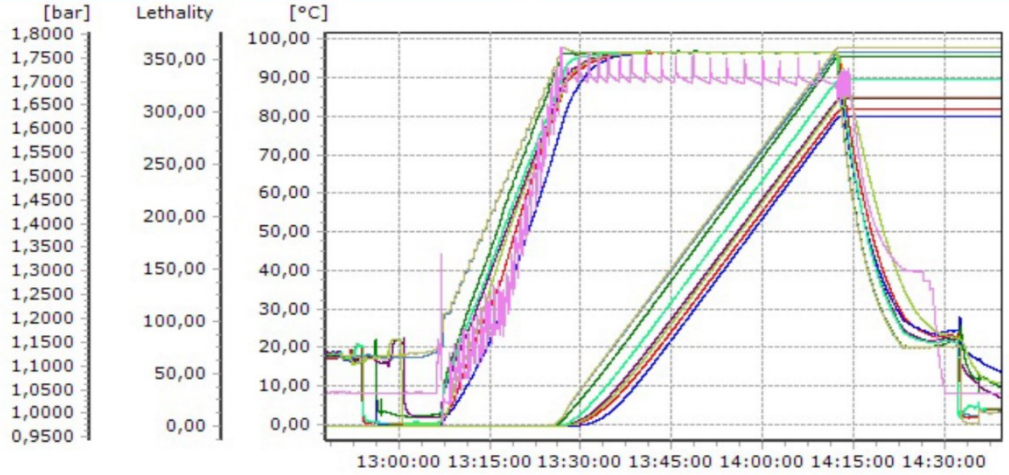
Retort treatment 2

A.

Operator:	Master	Time Zone:	(UTC+01:00) W. Europe Standard Time
Process:	96 °C 45 min	Vessel:	Autoklav
Session Start:	21.01.2021 12:47:57	Product:	Blomkål
Session Stop:	21.01.2021 14:39:47		
Session Name:	Blomkål CF 2		
Session Text:	N/A		

Validation Solutions

Graph



Sensor ID	Name	Validation Equipment	Description	Sample Rate 1/2 (hh:mm:ss)	Type	Sensor group
31517-1	Probe 1	13553	N/A	00:00:01/-	TC	N/A
29935-2	Probe 2	13553	N/A	00:00:01/-	TC	N/A
29932-3	Probe 3	13553	N/A	00:00:01/-	TC	N/A
31196-4	Probe 4	13553	N/A	00:00:01/-	TC	N/A
32790-5	Probe 5	13553	N/A	00:00:01/-	TC	N/A
31513-6	Probe 6	13553	N/A	00:00:01/-	TC	N/A
30064-7	Probe 7	13553	N/A	00:00:01/-	TC	N/A
29937-8	Probe 8	13553	N/A	00:00:01/-	TC	N/A
180681-64	Probe 9	13553	N/A	00:00:01/-	P	N/A

B.

Lethality Overview

Input parameter

Start Date :	21.01.2021
Ref. Temperature:	90,00 °C
Min. Temperature :	60,00 °C
Z-Value:	7,5 °C
D-Value:	1

Sensor lethality overview

Name	Description	Start Time	End Time	Max. Value
Probe 1 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	303,04
Probe 2 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	295,61
Probe 3 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	353,56
Probe 4 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	313,50
Probe 5 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	357,53
Probe 6 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	332,24
Probe 7 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	361,81
Probe 8 (F0) Lethality 1	L-Calculation	12:47:57	14:39:47	314,58

Figure 2. (A.) Shows the heating distribution, were probe 1, 2, 3, 4, 6, and 8 were inserted in the cauliflower. Probe 9 is showing the pressure. Probe 5 and 7 is showing the temperature of the water. When calculating the F-value for the average temperature of all the probes inserted in the cauliflower, a value of F=133 min. Also, the average temperature remained at 90-96-90 °C for 45.13 min. Comparing this for the first run, a difference of 37.8 seconds is found, meaning that the heating pattern was close to similar. (B) Shows the F-value for each probe (also called L-value as indicated here).

Table 1. The two treatments were not found to be sign. different (p=0.726) between the first and second run during the main heating period holding 90-96-90 °C.

Grouping info using the Tukey Method and 95 % Confidence			
Factor	N	Mean	Grouping
Run 1	2747	96.13	A
Run 2	2709	96.12	A

Appendix B. Temperature data for MW treatment

Typical MW profiles

The increase in temperature in the end of each run was due to the package expanded (because of pressure release), which moved the temperature probe out of the cold spot and out to warmer spots. Thus, the temperature of the cold spot reached its maximum at 660 s, before the cool-down phase.

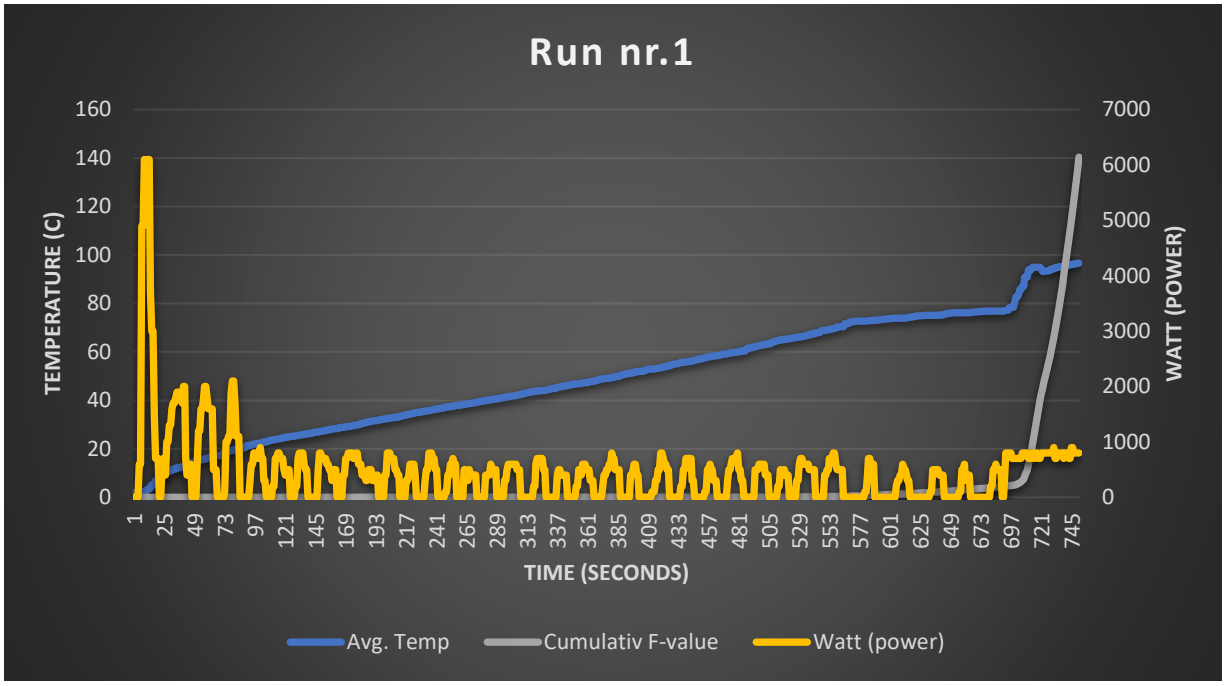


Figure 1. Illustrates the temperature increase for the cold spot of MW run nr.1: Total energy absorbed during the whole treatment was 342.5 kJ, F-value was a total of 0.004 min. The yellow plot shows the cycles of Watt introduced to the cauliflower.

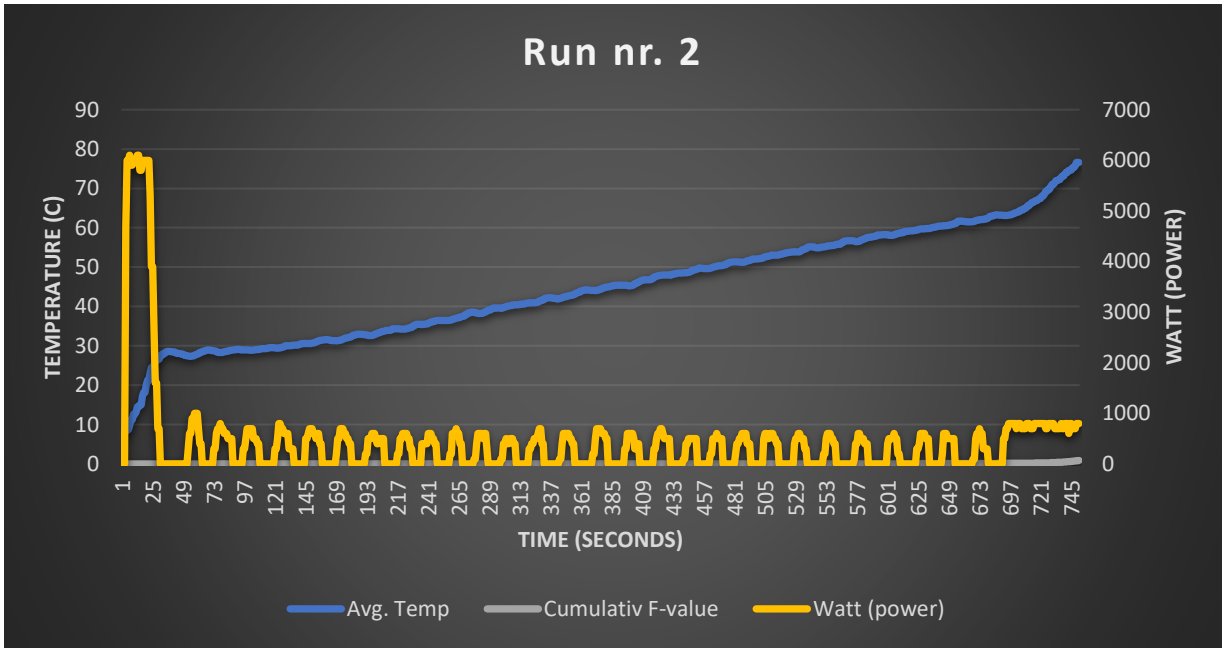


Figure 2. Illustrates the temperature increase for the cold spot of MW run nr.2: Total energy absorbed during the whole treatment was 339.1 kJ, F-value was a total of 0.000725 min. The increase in temperature in the end is the result of pressure release. The yellow plot shows the cycles of Watt introduced to the cauliflower.

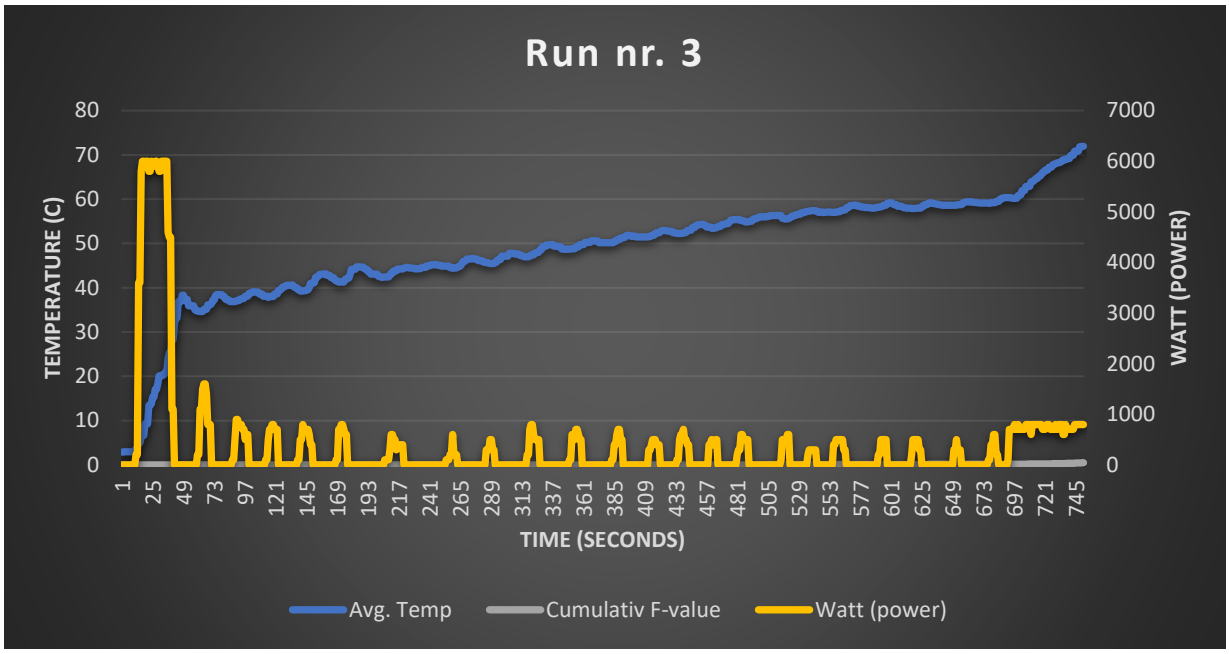


Figure 3. Illustrates the temperature increase for the cold spot of MW run nr.3: Total energy absorbed during the whole treatment was 287.8 kJ, F-value was a total of 0.00008225 min. The yellow plot shows the cycles of Watt introduced to the cauliflower.

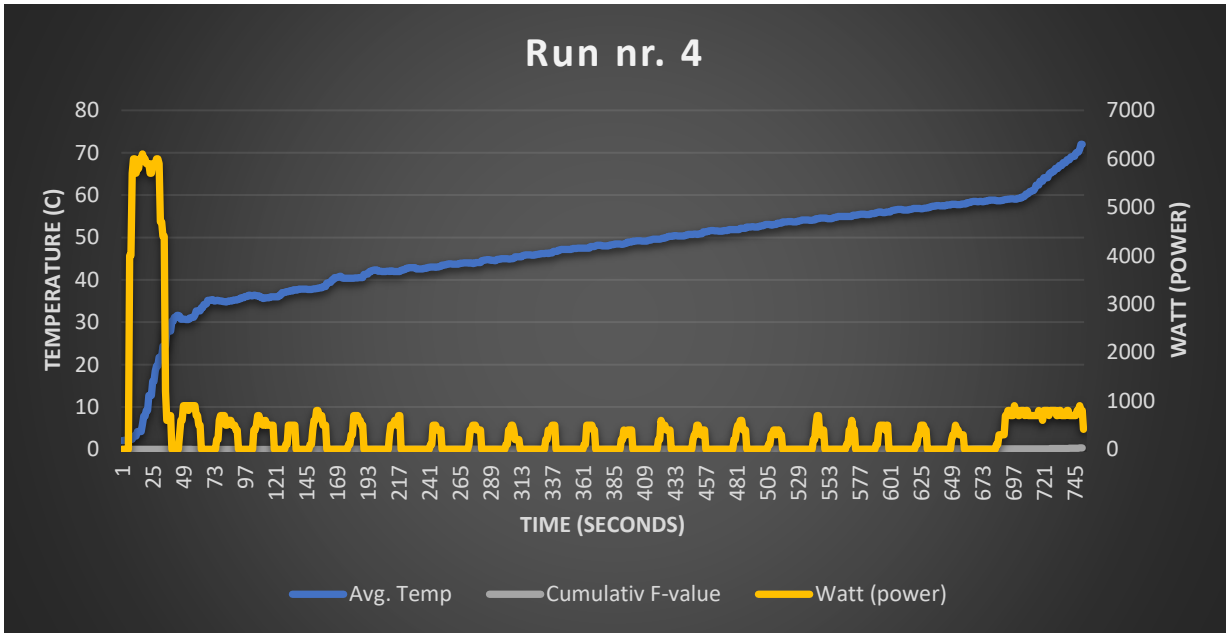


Figure 4. Illustrates the temperature increase for the cold spot of MW run nr.4: Total energy absorbed during the whole treatment was 319.1 kJ, F-value was a total of 0.00049 min. The yellow plot shows the cycles of Watt introduced to the cauliflower.

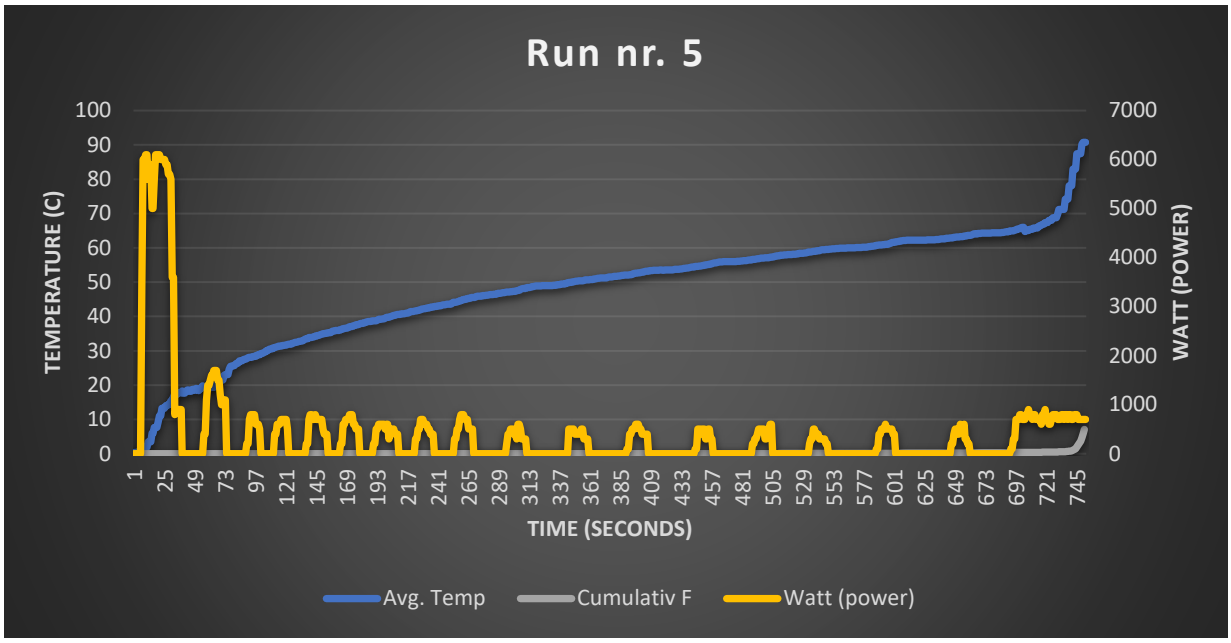


Figure 5. Illustrates the temperature increase for the cold spot of MW run nr.5: Total energy absorbed during the whole treatment was 323.4 kJ, F-value was a total of 0.0021 min. The yellow plot shows the cycles of Watt introduced to the cauliflower.

Summary of all 5 MW treatments

Table 1. Presents the energy absorbed (kJ) and the F-value for the cold spot during each run of MW treatment, and the average and SD for all together.

Run nr.	Absorbed kJ	Calculated F-value in cold spot (min)
1	342,5	0,004
2	339,1	0,0007
3	287,8	0,0082
4	310,1	0,0005
5	323,4	0,0021
Average	**320,58**	**0,0031**
SD	**22.45**	**0,003**

Table 2. Factor indicates nr of run, and mean is the average temperature achieved in the cold spot. N is measured through all MW processing, of a total of 750 s (12.5 min, included cool-down phase). Means that does not share a letter is significantly different.

Grouping info using the Tukey Method and 95 % Confidence			
Factor (Run nr.)	N	Mean	Grouping
1	751	49.82	A
3	751	48.73	A B
5	752	48.08	A B
4	751	46.65	B C
2	751	44.74	C

Appendix C. Summary of L*, a*, b* color values of all treatments.

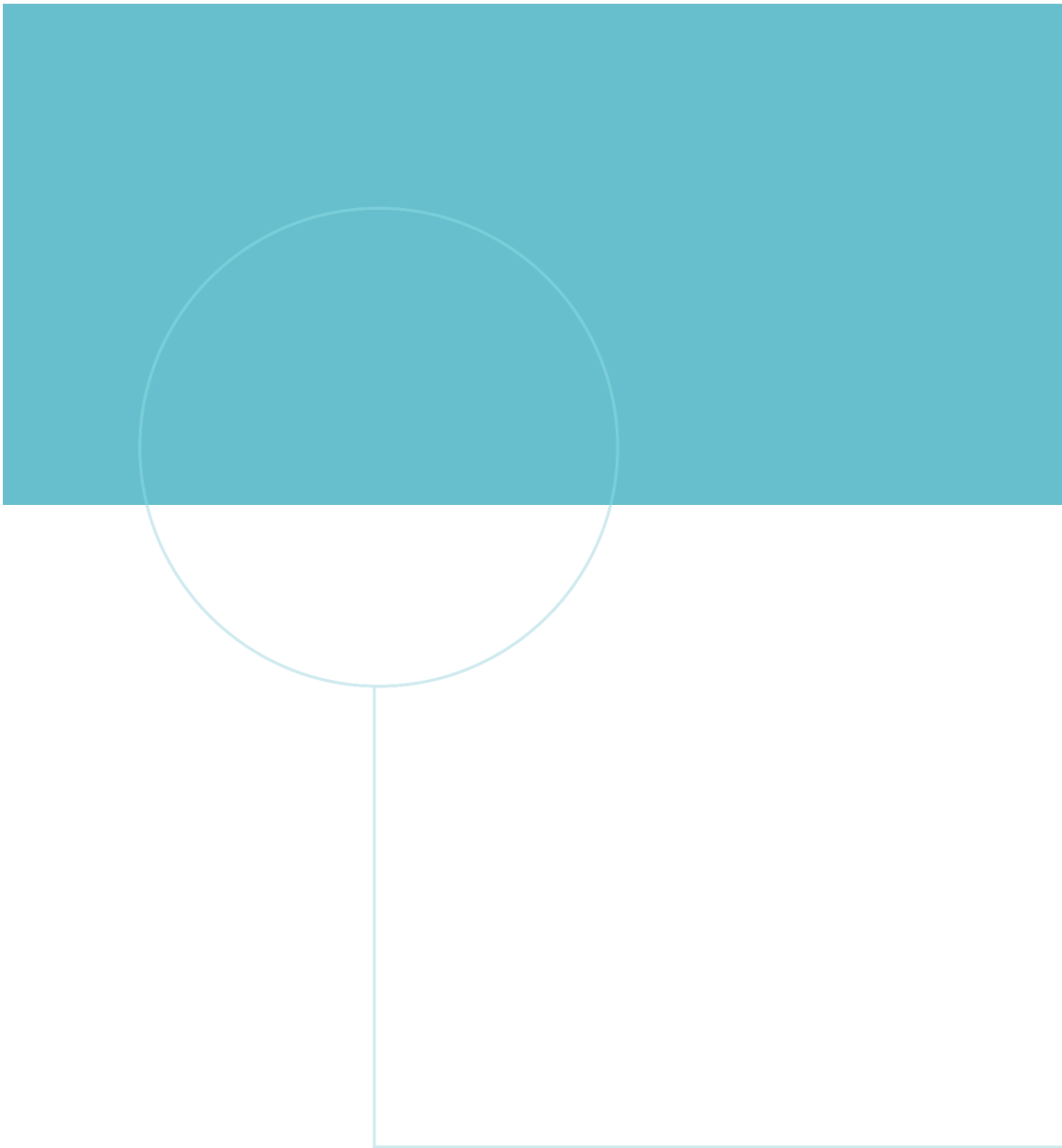
Table 1. The L*, a*, b* values * for the stem (A) and floret (B) of cauliflower was measured after HPP (400 and 600 MPa), microwave, and retort treatment. The samples were stored for up to 42 days cold storage (4 °C).

Color value	A. Stem							
	Storage (days)	Control	HPP				Retort	Microwave
		0.1 MPa	400 MPa		600 MPa		96 °C	320 kJ
		0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
*L**	*0*	79.4±2.2 ^{b}	79.8±2.2 $^{A, ab}$	79.5±2.6 $^{A, ab}$	80.5±2.0 $^{A, ab}$	79.2±2.0 $^{A, b}$	76.8±2.4 $^{A, c}$	81.1±2.2 $^{A, a}$
	14	nm**	79.5±2.0 $^{A, ab}$	79.5±1.6 $^{A, ab}$	79.2±2.4 $^{A, ab}$	78.5±2.4 $^{A, b}$	75.9±2.4 $^{AB, c}$	80.4±1.9 $^{A, a}$
	28	nm	79.5±2.4 $^{A, a}$	80.7±2.5 $^{A, a}$	80.4±2.2 $^{A, a}$	80.2±2.5 $^{A, a}$	74.9±2.5 $^{B, b}$	nm
	42	nm	79.2±2.6 $^{A, a}$	79.3±2.4 $^{A, a}$	80.0±2.3# $^{A, a}$	79.0±2.1 $^{A, a}$	71.5±2.3 $^{C, b}$	nm
*a**	*0*	-2.6±1.0 ^{a}	-2.9±0.9 $^{B, a}$	-2.8±1 $^{B, a}$	-2.6±1.1 $^{B, a}$	-2.8±1.3 $^{B, a}$	-0.9±0.8 $^{A, b}$	-2.2±0.7 $^{A, a}$
	14	nm	-1.7±0.8 $^{A, a}$	-1.2±1.1 $^{A, a}$	-1.5±0.8 $^{A, a}$	-1.4±0.6 $^{A, a}$	-0.3±0.9 $^{B, b}$	0.3±1 $^{B, b}$
	28	nm	-1.3±1.8 $^{A, a}$	-1.8±1.3 $^{A, a}$	-1.8±1.6 $^{A, a}$	-1.7±1.4 $^{A, a}$	0.3±1.1 $^{C, b}$	nm
	42	nm	-1.4±2.0 $^{A, ab}$	-1.0±2.0 $^{A, b}$	-2.7±1.2# $^{B, a}$	-1.2±1.9 $^{A, b}$	0.7±0.7 $^{C, c}$	nm
*b**	*0*	19.8±2.0 ^{a}	20.1±2.3 $^{AB, a}$	19.5±2.1 $^{A, a}$	20.0±2.5 $^{A, a}$	19.4±2.4 $^{A, a}$	20.0±2.1 $^{BC, a}$	18.8±2.2 $^{A, a}$
	14	nm	19.3±1.8 $^{AB, b}$	18.5±1.9 $^{A, bc}$	18.2±2 $^{B, bc}$	17.6±1.9 $^{B, c}$	21.5±1.9 $^{A, a}$	17.8±1.6 $^{A, bc}$
	28	nm	20.7±2.2 $^{A, ab}$	19.1±2.1 $^{A, bc}$	19.7±2.3 $^{A, abc}$	18.7±2.2 $^{AB, c}$	21.2±2.4 $^{AB, a}$	nm
	42	nm	19.0±3.0 $^{B, a}$	19.3±2.2 $^{A, a}$	20.2±2.7# $^{A, a}$	19.0±2.6 $^{A, a}$	19.9±1.4 $^{C, a}$	nm

Color value	B. Floret							
	Storage (days)	Control	HPP				Retort	Microwave
		0.1 MPa	400 MPa		600 MPa		96 °C	320 kJ
		0 min	*2 min*	*5 min*	*2 min*	*5 min*	*45 min*	*10 min*
*L**	*0*	81.2±3 [b]	82.6±1.9 [A, ab]	81.5±2.5 [A, ab]	82.9±1.8 [A, a]	81.7±2.2 [A, ab]	78.4±2.5 [A, c]	82.4±3.1 [A, ab]
	14	nm**	82.4±2.1 [A, b]	81.8±1.8 [AB, b]	82.8±1.6 [A, b]	82.0±2.1 [A, b]	76.7±2.5 [B, a]	82.9±2.4 [A, b]
	28	nm	82.6±1.9 [A, b]	83.0±1.9 [B, b]	82.6±1.8 [A, b]	82.7±2.6 [A, b]	77.2±1.4 [AB, a]	nm
	42	nm	82.2±1.9 [A, b]	81.7±2.0 [A, b]	82.8±2.0 # [A, b]	81.5±2.3 [A, b]	73.2±2.7 [C, a]	nm
*a**	*0*	-0.3±1.2 [b]	-0.7±0.7 [B, bc]	-0.5±1.1 [A, bc]	-1.0±0.7 [B, c]	-0.7±1.0 [A, bc]	1.7±1.1 [A, a]	-0.3±0.9 [A, bc]
	14	nm	0.2±0.9 [A, b]	0.7±0.7 [B, b]	0.2±0.7 [A, b]	0.2±0.5 [BC, b]	2.4±1.2 [B, a]	2.2±1.4 [B, a]
	28	nm	0.6±1.6 [A, b]	0.1±1.5 [AB, b]	-0.0±1.3 [A, b]	-0.2±1.0 [AB, b]	3.3±1.2 [C, a]	nm
	42	nm	0.6±2 [A, b]	0.9±2.3 [B, b]	-1.0±0.8 # [B, c]	0.7±1.6 [C, b]	3.2±1 [C, a]	nm
*b**	*0*	28.7±4.5 [a]	26.7±4.2 [A, ab]	26.8±4.7 [A, ab]	25.2±3.3 [A, b]	26.2±3.8 [A, b]	27.3±2.9 [A, ab]	25.1±4.4 [A, b]
	14	nm	27.1±4 [A, ab]	24.5±3.6 [B, c]	24.9±3.2 [A, bc]	24.8±3.2 [AB, bc]	27.5±3.9 [A, a]	26.7±4.6 [A, abc]
	28	nm	27.6±4.1 [A, a]	24.9±3.5 [AB, bc]	24.9±3.5 [A, bc]	23.5±3.6 [B, c]	26.5±3.3 [A, ab]	nm
	42	nm	26.6±4.3 [A, a]	25.5±3.6 [AB, a]	25.5±4.1# [A, a]	25.3±3.4 [AB, a]	25.9±3.0 [A, a]	nm

Each color value (L, a*, b*)) in the table is the mean ± standard deviation from processing parallels (n=3). The capital letters (A, B, and C) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ($p<0.05$, multiple Tukey's adjusted analysis of variance) between different treatments and days (same row).

**Not measured. # Uncertain value, less samples due to hull in package during storage.



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