Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsplanering,
trädgårds– och jordbruksvetenskap

Healthier vegetables through temperature stress?

- A review of *Brassica oleracea*

Anna Enocksson

Självständigt arbete vid LTJ-fakulteten, SLU

Kandidatarbete i Biologi (15 hp), Hortonomprogrammet, Alnarp 2013

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsplanering, trädgårds- och jordbruksvetenskap

Healthier vegetables through temperature stress? – A review of *Brassica oleracea*

Nyttigare grönsaker med hjälp av temperatur stress? – En studie av *Brassica oleracea*

Anna Enocksson

Supervisor: Assistant Professor Lars Mogren, SLU, Department of Biosystems and Technology

Examiner: Professor Marie Olsson, SLU, Department of Plant Breeding

Credits: 15 hp (hec)

Level: G2E

Course title: Kandidatarbete i biologi

Course code: EX0493

Programme/education: Hortonomprogrammet, Horticultural Science Programme

Place of publication: Alnarp, Sweden

Year of publication: 2013

Cover pictures: Different forms of subspecies of *B. oleracea*. Photography: Anna Enocksson

Title of series: Självständigt arbete vid LTJ-fakulteten, SLU

Online publication: http://stud.epsilon.slu.se

Keywords:

Health, nutrition, quality, crucifers, Brassica oleracea, broccoli, Brussels sprouts, cabbage, cauliflower, kale, temperature, heat, antioxidants, phytochemicals

Responsible department:

Swedish University of Agricultural Sciences (SLU)
Faculty of Landscape Planning, Horticulture and Agricultural Sciences
Department of Biosystems and Technology

Preface

During my work on this report I went through both a long period of sickness and a move abroad. It was a very bumpy road and I am very grateful to my supervisor Lars Mogren (SLU) who helped me sort out my thoughts and find the concept and form for this report. Thanks to his help I was able to dive into an area which requires knowledge in both plant physiology, postharvest processes, human nutrition and much more. I owe both him and my family and friends great thanks for their help and support which has been vital for the processes of writing this report.

Thank you all dearly.

Anna Enocksson

Stuttgart, June 2013

Sammanfattning

Målet med detta arbete är att sammanfatta den information som i dagsläget finns angående hur temperatur påverkar grönsaker fysiologiskt när det kommer till deras hälsofrämjande respektive kvalitetsnedsättande egenskaper. Arbetet inleds därför med en närmare beskrivning av vad som i detta fall menas med just hälsofrämjande och kvalitetsnedsättande egenskaper och går sedan vidare med att beskriva hur olika underarter av *Brassica oleracea*, det vill säga grönsaker så som broccoli, blomkål, brysselkål och grönkål, påverkas av temperatur. *B. oleracea* valdes framför andra arter eftersom arten innehåller flera underarter av kommersiellt intresse och därmed utgör en bred bas även om enbart en art avhandlas. Flera av underarterna har även rönt intresse inom populärvetenskapen på senare år och framhävts som mycket nyttiga vilket gör dem intressanta för detta arbete. Fokus gällande temperatur ligger i arbetet främst på temperaturbehandlingar under processning och tillagning då detta är det som är mest undersökt, men även mer allmän temperaturstress behandlas både gällande pre- och postharvestförhållanden. De slutsatser som kan dras baserat på detta arbete är att grönsaker fysiologiskt påverkas kraftigt av temperatur, och att temperaturprocessning minskar den sensoriska kvaliteten hos *B. oleracea* samtidigt som det kan höja dess hälsofrämjande effekt, så länge rätt tillagningsmetod väljs. Innehållet av dessa hälsofrämjande ämnen varierar dock stort mellan genotyper av samma underart vilket innebär att klara definitioner är nödvändiga.

Abstract

The objective of this report is to present a review of the studies which has been carried out up to days date concerning how temperature affects vegetables physiologically when it comes to their health inducing as well as quality reducing features. The essay therefore starts off with defining what is meant by the terms health promoting and quality reducing features. It then moves on to describe how different subspecies of *Brassica oleracea*, that is to say vegetables such as broccoli, cauliflower, Brussels sprouts and kale, is affected by temperature. *B. oleracea* was chosen because the species contains several commercially interesting subspecies and therefore provides a broad field even though only one species is assessed. Several of the subspecies have also been of special interest in the popular science in later years and have been said to be very healthy which makes them interesting for this thesis. Concerning temperature, focus is put mainly on temperature treatments during processing and cooking since this field has been studied more thoroughly. However, general temperature stress is also being discussed, both concerning pre- and postharvest conditions. The conclusions which can be made based on this report are that vegetables are physiologically very affected by temperature, and that temperature processing reduces the sensory quality of *B. oleracea* whereas it may actually enhance its health promoting effects, if the right cooking method is chosen. However, the content of these health promoting compounds varies greatly between genotypes within one subspecies which makes clear definitions essential.

Table of contents

1. Introduction

1.1 Background

Both fruits and vegetables are necessary parts in a healthy diet. They have been found to prevent, and in some cases help to treat (Evangelou *et al*, 1997), several different chronic and degenerative diseases (Livsmedelsverket, 2012). Many of these diseases are believed to partly be caused by oxidative stress which is one of the reasons vegetables (and fruits) can help to prevent disease, since they are the main source of antioxidants in the diet (Ames *et al*, 1993). In recent years it has been established that antioxidants alone do not seem to prevent diseases such as cancer but instead there seems to be a more complex interaction pattern where other compounds are also involved, especially other phytochemicals in the case of *Brassica oleracea* (Temple, 2000). The fact that a regularly low consumption of fruit and vegetables is related with higher risks of degenerative diseases such as cancer and cardiovascular diseases (Liu, 2003) still stands though, even if the cause of this is currently partially unknown. When considering that these are the two major causes of death in most industrialized countries (Liu, 2003) it becomes clear how important every mean of prevention becomes.

The Food and Agriculture Organization of the United Nations (FAO) states that the production quantity of Brassica vegetables in 2010 amounted to about 84,5 million tonnes in the world, making up 8% of all primary vegetables grown in the world that year (FAO, 2013). That quantity is about seven times the amount of rye produced in the same year. The gross value of the production amounted to almost 30 billion dollars worldwide in 2010 where Poland, Russia, United Kingdom, Belarus, Belgium, Germany and Spain are some of the European countries with the largest gross production values.

1.2 Objectives

The objectives of this report are to review what current literature writes about how temperature stress, mainly heat, affects the health promoting and quality reducing factors and compounds of *Brassica oleracea*. Based on this, more general conclusions are made considering vegetables in general. The questions that this report aims to answer are:

- How does temperature stress affect vegetables physiologically?
- How does temperature (mainly heat) affect the content of health promoting compounds in *B. oleracea*?
- How does temperature (mainly heat) affect the quality of *B. oleracea*?

1.3 Scope and limitations

Due to the time constraints of a BSc thesis project, focus had to be put on one or a few vegetables and from these more generalized assumptions could hopefully be made. During the preparation for this report different vegetables were considered but in the end *Brassica oleracea* was chosen since it contains several different subspecies which have been mentioned recently in popular science due to their allegedly high nutritional and health values. They are also being grown in several different parts of the world, Asia and Europe being some of the largest producers, but Kenya and USA are examples of other countries with a noticeable production (FAO, 2013). This makes them interesting from an international perspective as well. Within the species some of the most common vegetables were chosen and minor vegetables such as kohlrabi and romanesco broccoli (also known as Roman cauliflower) was not included even if the latter belongs to the Botrytis Group together with cauliflower. This was based on the literature available.

The original objective of this report was to study the effects of preharvest temperature stress on the nutritional quality of *B. oleracea*. However, since there is lacking material on the effect of preharvest temperature stress this report mainly focuses on temperature processing (different cooking methods) and postharvest temperature stress, but references to preharvest effects are made where relevant material could be found.

Many studies have focused on the nutritional value of *B. oleracea* after thermal processing, but few have considered the overall quality. This report therefore includes not only nutritional quality, but also other aspects such as color and taste (bitterness). Focus lies on the sensory quality as perceived by the consumer and not on shelf life and other similar aspects of quality. Texture is also considered as a quality aspect, but is generally not mentioned in the results since it is common knowledge that vegetables which are boiled or cooked too long become softer and lose their crispy texture.

2. Materials and methods

2.1 Information gathering

For this report some different databases were used:

- Google Scholar
  - This database was only used in the very beginning for creating an overview on the theme and to see how the report should be limited based on what kind of studies were available on the subject.
- Web of Knowledge
  - This database was the one which was used the most due to its wide range of subjects, well needed for this reports since it depends strongly on different fields of study such as Food Chemistry, Pharmaceutical and Medical Science and Botany.
- Science Direct
  - This database which only carries Elsevier articles was used to narrow down the results from Web of Knowledge and to find new key words to bring back to Web of Knowledge were articles from more publishers could be found.
- ScienceResearch and SCOPUS
  - These databases were used for finding additional articles which proved necessary later on in the working process. Web of Knowledge could not be used at that time since the searches were done at a German University which did not have a license for it.

2.2 Chosen materials

Most of the information used in this review comes from primary sources, mainly articles published in different journals which were found by using the databases stated in 2.1. However, there were a few cases when the primary sources were not accessible (though existing) or in a foreign language and in those cases secondary sources were used. Some of the references to Kurilich *et al* (1999) made in 3.2 Results are secondary sources since several of the originals are too old to find in e-libraries and Kurilich *et al* (1999) has written a good review explaining the positive effects of some compounds in *B. oleracea*. Cases like these have been avoided as far as possible and since this report is meant to review more current studies articles were chosen before books if a choice was possible.

2.3 Definitions

Health promoting compounds

There are many phytochemicals which have been said to promote health through prevention of diseases and so forth. This report focuses on some of them such as ascorbic acid, carotenoids and tocopherols, (poly)phenols and glucosinolates. Their functions are elaborated on in 3.2.

Quality and quality aspects

Quality is something highly subjective and the perception of it will often vary between people but it can generally be grouped into four categories (Dixon, 2007):

- *Sight* such as color, size and shape as well as potential visible defects
- *Texture*
- *Flavor* and *smell*
- *Invisible aspects* such as nutritional value and added pesticides

This report only discusses consumer quality and since literature is scarce on the effects of temperature stress on these factors it will not be discussed for each subspecies and focus is put mainly on color and taste. Color is discussed by using terms such as greenness, color saturation and chlorophyll content, assuming that a higher value equals a vegetable more attractive to consumers (cauliflower excluded). Concerning taste bitterness is considered to negatively affect the quality and this is measured by the amount and composition of glucosinolates, such as sinigrin and progoitrin in the case of Brussels sprouts (van Doorn *et al*, 1998).

2.4 Glossary

Antioxidants (antioxidant phytochemicals)

Antioxidants are molecules with the capacity to inhibit the oxidation of other molecules by using different strategies (enzymatic and non-enzymatic). Oxidation is a natural process of aerobic metabolism in cells but can also lead to a production of free radicals which can later harm or even destroy the cells (Sies, 1997). Examples of antioxidants are glutathiones, thiocyanates and polyphenols. Vitamins (a group of vital nutrients which cannot be synthesized in the human body) and their precursors, such as ascorbic acid, carotenoids and tocopherols also have antioxidant capacity (Singh *et al*, 2007).

Ascorbic acid/Ascorbate

Ascorbic acid is a form of vitamin C. L-ascorbic acid is the form that exists in nature and D-ascorbic acid is a synthetic form. Ascorbate is the anion of ascorbic acid.

Biosynthetic pathways

All compounds discussed in this report have rather complex chemical structures and are the result of different biosynthetic pathways. Examples of this are how glucosinolates are synthesized from amino acids and glucose, or how ascorbic acid is synthesized from glucose. Many of these pathways are sometimes linked through shared intermediaries, or through more direct ways, where for example lycopene is not only a carotenoid of its own, but also an intermediary in the pathway of α- and β-carotene.

Carotenoids

Carotenoids are organic pigments which can be divided into two different groups: 1) xanthophylls such as lutein and zeaxanthin which contain oxygen, and 2) carotenes such as α-, β-carotene and lycopene which do not contain oxygen. They are antioxidants and thus prevent oxidation.

Crucifers

Crucifers are vegetables belonging to the family Brassiceae, also known as Cruciferae (meaning “cross-bearing”, concerning the shape of the flowers of this family). *Brassica* is one of the genera in this family and *B. oleracea* one of the species in the genus *Brassica*.

Flavonoids/Flavonols

Flavonoids are very common secondary metabolites which functions can include flower pigmentation and UV filtration in higher plants. They are often found in the human diet. Flavonoids are a structural class in which the compounds have a common C_6-C_3-C_6 structure. Examples of flavonoids are quercetin and kaempferol. Flavonols is a subgroup of flavonoids, created based on chemical structure, which is also very commonly found in the human diet.

Glucosinolates

Glucosinolates are common secondary metabolites of plants of the order Brassicales and are derived from glucose and an amino acid, containing sulfur and nitrogen. There are many different types of glucosinolates and they are divided into groups depending on from which amino acid they were derived.

Hydroxycinnamic acids (hydroxycinnamates)

Hydroxycinnamic acids are polyphenols with the same basic chemical structure (C6-C3 skeleton). There are several different types of hydroxycinnamic acids but among the phytochemicals, substances such as caffeic acid, sinapic acid, chlorogenic acid and coumaric acid can be found. Many of them are considered active antioxidants.

Phytochemicals

Phytochemicals are chemical compounds that occur naturally in plants, no matter what their functions are. However, the term is usually used for compounds which are believed to hold biological significance without being nutrients, such as antioxidants and this is the meaning of the term in this report.

Polyphenols

The term polyphenols is a structural classification for larger phenol molecules consisting of several phenol units. A phenol unit consists of a phenyl group ($-C_6H_5$) bound to a hydroxyl group (-OH).

Preharvest/Postharvest/Processing

All processes, treatments and interventions which are taking place before the vegetable is harvested are considered to be preharvest processes, whereas those taking place after harvest, during for example cooling (after harvest) or storage, are postharvest processes. Processing comes after the postharvest period where the vegetables are prepared for consumption by different cooking and freezing methods, through which the metabolism stops due to the cells no longer being alive. Changes in the chemical composition during or after processing are therefore mainly due to the degradation of larger molecules into smaller molecules (other substances). This makes it possible for the content of some substances to increase if they are the degradation products of other chemical substances in the plant.

Tocopherols

Tocopherols are organic compounds with vitamin E activity, consisting of methylated phenols. They have antioxidant effects and exist in four different forms: α-, β-, γ- and δ-tocopherol where α- and γ-tocopherol are most common in *B. oleracea* (Kurilich *et al*, 1999).

3. Results

3.1 *Brassica oleracea*

Brassicaceae, the plant family more commonly known as crucifers to which *Brassica oleracea* belongs, is grown (Zietz *et al*, 2010) and consumed worldwide (Podsędek, 2007). In recent years the consumer demand for better quality, or retention of quality postharvest, has increased (Dixon, 2007). This change started out in the 1950's as the "quick-frozen fresh produce" hit the markets and brought with it higher quality standards. These have since been developed and for growers it has become a leading goal to provide high quality cruciferous vegetables to their customers.

3.2 Nutritional value and quality of *B. oleracea*

Crucifers generally contain high amounts of phenolics and glucosinolates, making them a good source of antioxidants (Moreno *et al*, 2006). These compounds are commonly considered to be health improving, due to the reports on their preventive effects on cardiovascular diseases and on cancer (Moreno *et al*, 2006). However, there have also been reports stating anti-nutritional properties among the crucifers due to polyphenols, glucosinolates, tannins and other compounds which have reduced the growth in animals and disturbed hormone levels (Griffiths *et al*, 1998).

Brassica provides a wide source of health affecting compounds and is considered a staple food (Jahangir *et al*, 2009) which proves its value and the need to understand its affect on human health. The health benefits associated with *Brassica* are according to Dixon (2007) related to their glucosinolate content. There are more than 100 different forms of glucosinolates in brassicas, all with a common basic structure and a variable side chain as can be seen in figure 1. The side chain then decides which group the glucosinolate belongs to, the aliphatic, indole or aromatic group (Mithen *et al*, 2000). Several indole glucosinolates are considered to have anti-carcinogenic properties (van Doorn *et al*, 1998) and the alkyl glucosinolate glucoraphanin is also believed to possess health promoting features (Schonhof *et al*, 2007b). The glucosinolate sinigrin has been shown to be partially responsible for the bitter taste in both cooked Brussels sprouts (van Doorn *et al*, 1998) and cauliflower (Engel *et al*, 2002). Isothiocyanates in cruciferous vegetables are suspected to block the enzyme thyroid peroxidase and thereby reduce the thyroid function and the production of thyroid hormones, if consumed in too high amounts (WH Foods, 2001). Though it has not been proven to be the case in humans so far, it has been shown to have this effect on animal livestock (Cornell University, 2013) and is therefore sometimes considered an anti-nutrient.

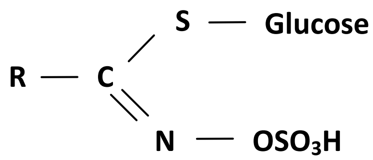

Figure 1. The general structure of a glucosinolate with R being a variable side chain of hydrocarbons. Adapted from Mithen *et al* (2000).

Crucifers contain several carotenoids as well as tocopherols and ascorbic acid that can act both as vitamins and as antioxidants, which may make them able to prevent different degenerative diseases (Kurilich *et al*, 1999). As earlier stated, much of the health benefits coming from human consumption of crucifers is said to be due to the glucosinolates and their abilities to reduce the risk of cancer. That is however not the only benefit which is brought by consumption of crucifers. They are also a good source of vitamin antioxidants which may help to protect against cancer as well as other degenerative diseases. Kurilich *et al* (1999) tested the amount of tocopherols, carotenes and ascorbic acid (in the shape of its anion ascorbate) in broccoli, Brussels sprouts, cauliflower, kale and cabbage focusing mainly on broccoli due to its stronger economic importance and higher consumer preference.

Kurilich *et al* (1999) has shown that based on concentration it can be established that the most important vitamins in the five tested subspecies of *B. oleracea* are β-carotene (pro-vitamin A), α-tocopherol (vitamin E) and ascorbate (vitamin C). Among the subspecies, ascorbate was the most abundant vitamin antioxidant based on concentration. The study could also conclude that there is an indication that levels of tocopherol, carotenoids and ascorbate could be enhanced simultaneously since they do not seem to affect each other negatively.

Apart from the antioxidant effect of the individual vitamins there has also been reports about them showing synergistic activity. However, it is to be stated that the vitamin content was found to greatly vary depending on the genotype within the subspecies. Kurilich *et al* (1999) reported this to be the case with broccoli where they tested 50 different accessions, but the importance of the genotype has been reported for more *B. oleracea* subspecies such as kale (Zietz *et al*, 2010; Schmidt *et al*, 2010). It is therefore suspected to be a very important factor which needs to be taken into consideration when working on a breeding program with the aim to enhance the health impact of the chosen subspecies.

3.3 *B. oleracea* and temperature

3.3.1 General pre-, postharvest and processing temperature effects

Preharvest

Vegetables are in general sensitive to extreme environments and to drastic changes in their environment and have narrow temperature ranges in which they can grow well. Unlike people and animals they are not able to simply leave an unfavourable environment so instead they have found other defence mechanisms. These defence mechanisms do not come without costs for the plant so the result is often that plants subjected to stress have lower yields (Abou-Hussein, 2012), but in the case of heat stress these defence mechanisms make it possible for the plant to minimize the damage caused to it and protect its cellular homeostasis with the help of for example heat stress proteins (Kotak *et al*, 2007) or low temperature stress proteins (Guy, 1999). Both in the case of low and high temperatures plants show a possibility to acclimate themselves if given the opportunity. If plants are subjected to non-lethal extremes this will induce cold acclimation in the case of cold or an acquired thermotolerance in the case of heat, which makes the plant able to shortly after withstand temperatures which would previously have proven lethal for it (Guy, 1999). The response a plant gives to heat stress is a complex process with intricate signalling pathways and the researchers are only beginning to understand how it is all connected (Kotak *et al*, 2007). Different vegetables are differently vulnerable to more extreme preharvest temperatures and Abou-Hussein (2012) summarizes current literature on the topic and notes that high temperatures can for some species lead to improved germination wheras it for others causes decreasing growth rates and makes them more susceptible to certain diseases. When the temperature rises too high too quickly for a plant to handle the protoplasts in its cells are destroyed which then leads to the death of the whole cell. However, it is important to note that what is meant by high temperatures here is temperatures higher than 25°C, and destruction generally tends to occur at temperatures around 45-55°C.

Though biotic factors, such as mechanical injuries, are considered major stress-inducers there are also several abiotic stress factors which can affect the production of metabolites. In this essay only temperature stress and thermal treatments will be discussed, though in reality high temperatures preharvest often lead to drought which can affect the metabolism and the production through different biosynthetic pathways. Temperature can also act together with radiation as a trigger for biosynthetic pathways such as for flavonoids, a compound which among other functions can act as flower pigments (Schonhof *et al*, 2007a). Flavonols, a subgroup of flavonoids, generally increase when plants are subjected to lower temperatures during growth (Neugart *et al*, 2012). Glucosinolate content is somewhat dependent on both temperature and radiation and the relationship between the three differs for each type of glucosinolate (Schonhof *et al*, 2007a). Seasonal variations between different types of glucosinolates (aliphatic, indole and aromatic) have also been observed (Cartea *et al*, 2008).

Postharvest

When subjected to postharvest treatments involving high temperatures a lot of physiological processes can be affected in the plant. Some of the effects can be inhibition of hormone production, especially ethylene at about 35°C, together with inhibition of ageing (senescence) and ripening processes (Lurie, 2006). The plant defence can also be induced by high temperatures, which may lead to the production of more secondary metabolites acting as defence agents. In some cases new synthesis of these secondary metabolites are protective actions induced by the heat treatments, and sometimes they are a negative side effect, depending on the compounds being produced. Too high temperatures can also give rise to external and/or internal damage on the vegetables which reduces their quality (Lurie, 2006).

Temperature processing can slightly decrease the amount of carotenoids in crucifers (Gebczynski & Lisiewska, 2006). The foremost effect is however a large decrease in the ascorbic acid content after boiling the crucifers, something that has been reported for both kale, Brussels sprouts, broccoli and white cauliflower (Gebczynski & Lisiewska, 2006). Boiling has also been found to severely decrease the amount of antioxidants due to the loss of polyphenols (Gebczynski & Lisiewska, 2006; Sikora *et al*, 2008). Freezing of raw crucifers on the other hand has so far been proven to be one of the best ways of preserving its nutritional benefits (Gebczynski & Lisiewska, 2006). The polyphenols are thereby preserved better and have even shown to increase as a stress response (Sikora *et al*, 2008).

3.3.2 Broccoli (*Brassica oleracea* Italica Group)

Pellegrini *et al* (2010) has tested different cooking methods (boiling, microwaving, basket and oven steaming) to see how they affect the phytochemical concentration and antioxidant capacity of different frozen and raw *B. oleracea* subspecies. They have shown that fresh broccoli actually becomes greener through boiling, even though a higher concentration of the chlorophyll molecule pheophytin α was detected, compared to other cooked products. Pheophytin α is a degradation product of chlorophyll (caused by cooking) which results in loss of the fresh greenness. The authors therefore argue that the actual increase in greenness in this case is probably due to a change in surface reflecting properties, possibly together with formation of other degradation products with a green colour. Fresh broccoli which was microwaved did instead lose greenness. Frozen broccoli which was cooked did in general keep its greenness and colour better, something Pellegrini *et al* (2010) believes could be due to the blanching process which is has been subjected to before freezing. The blanching could among other things have resulted in a thermal inactivation of enzymes and reduced oxygen content. This would have decreased the degradation of chlorophylls and made the pigments more stable after cooking, according to the hypothesis. The choice of cooking method is proving to be important when it comes to the preservation and even increase of some phytochemical compounds. As already stated, pheophytin α has been shown to increase through most cooking method and the same goes for the total glucosinolate content in fresh broccoli which is increased mainly by the two steaming treatments. In the frozen broccoli the total glucosinolate content instead decreases no matter which cooking method used. This Pellegrini *et al* (2010) believe is also due to the previous blanching and cold storage since it could have softened the vegetable matrix and caused losses of the glucosinolates.

Different cooking methods affect the carotenoid content differently in fresh and frozen broccoli. In fresh broccoli steaming methods did not affect the content at all, whereas microwaving caused a significant decrease of both lutein and β-carotene. Boiling also led to a significant decrease in lutein content. However, Pellegrini *et al* (2010) mention that these results do not confirm some previous findings where instead an increase in carotenoid content was shown, but there are other reports also showing decreasing amounts. When it comes to the frozen broccoli all the cooking methods caused a decrease of both lutein and β-carotene. The reason is believed to be the same as for the glucosinolate content.

Another phytochemical compound which was tested by Pellegrini *et al* (2010) is the ascorbic acid content. This was reported to decrease significantly in both fresh and frozen broccoli no matter which cooking method was used. However, for fresh broccoli the reduction was greatest when using the microwave method whereas for frozen broccoli the microwave method gave the best retention of ascorbic acid and instead it was the boiling which caused the greatest loss.

The major phenolic acid present in fresh broccoli was according to Pellegrini *et al* (2010) sinapic acid, followed by chlorogenic and coumaric acid, all different phenolic acids with antioxidant activity. The major flavonoid was quercetin. The different cooking methods affected the concentration of these substances in fresh broccoli very differently – boiling resulted in a decrease in all but the chlorogenic acid, and both basket steaming and microwaving resulted in significant losses of all substances, whereas oven steaming increased the amount of all the substances. The increased amounts of chlorogenic acid is suggested to be a result of cooking changing chemical structures, disrupting the interactions between chlorogenic acid and the polysaccharide part of fibers in the vegetable. The authors also believe that the effect of oven steaming could be due to inactivation of oxidative enzymes or the caused non-direct contact with water. This could mean that the substances are easier to catch in the tests, meaning that results showing higher amounts after cooking do not necessarily mean that the amounts of the substances have increased, but that they might simply have become easier to detect in tests. Frozen broccoli reacted differently from fresh and basket steaming was proven to be the best method which increased the phenolic compounds. Microwaving also proved to be good for retaining the compounds and increasing chlorogenic acid and kaempferol. Oven steaming and boiling both had severe negative effects on the concentrations of the compounds.

Pellegrini *et al* (2010) also determined the Total Antioxidant Capacity (TAC) which increased in fresh broccoli which was boiled or oven steamed, whereas the results from microwaving and basket steaming seem very dependent on how the method is carried out and which TAC assay is used. For frozen broccoli all cooking methods resulted in losses of TAC except for microwaving which kept the value stable. This negative effect on the nutritional quality is according to the authors the result of blanching and freeze storage which would cause disruptions of the cell membranes affecting several antioxidants.

3.3.3 Cauliflower (*Brassica oleracea* Botrytis Group)

Boiling and steaming changes the color of fresh cauliflower whereas microwaving helps keeping it stable. The color of frozen cauliflower was not much affected by any of the cooking methods, which the authors consider to be due blanching having a stabilizing effect.

Cauliflower does not contain enough carotenoids or chlorophyll for it to play any important nutritional role (Podsędek, 2007) and has also proved to contain only low amounts of glucosinolates compared to the other subspecies of *B. oleracea* (Pellegrini *et al*, 2010). However, glucobrassicin and glucoiberin are the major forms to be found.

Pellegrini *et al* (2010) showed that steaming has a good effect on increasing or retaining the glucosinolate levels in fresh cauliflower whereas boiling and microwaving decrease the amounts. The negative effect produced by microwaving goes against the results seen in fresh broccoli and Brussels sprouts and could mean that the same phytochemical group (glucosinolates) is affected differently in different vegetables or tissues. In frozen cauliflower a significant decrease of the glucosinolate levels was detected after boiling whereas microwaving led to a significant increase, and steaming resulted in no change.

Fresh cauliflower has a relatively high amount of ascorbic acid compared to broccoli and Brussels sprouts. All cooking methods resulted in significantly decreased levels in both fresh and frozen cauliflower and microwaving gave the greatest decrease in fresh cauliflower.

All cooking methods resulted in decreased total polyphenol levels for fresh cauliflower except oven steaming which retained the level (Pellegrini *et al*, 2010). The frozen cauliflower showed a similar pattern with retention being evident when using microwaving or basket steaming, the other methods resulted in a decreased level of total polyphenols.

In fresh cauliflower the Total Antioxidant Capacity (TAC) was the highest when steaming methods were used and lowest after microwaving (Pellegrini *et al*, 2010). Most cooking methods showed no significant effect on the TAC of frozen cauliflower, but boiling did result in significant decreases due to the loss of ascorbic acid and polyphenols.

Engel *et al* (2002) has shown that the major compounds responsible for the bitter taste of cooked cauliflower are the glucosinolates sinigrin and neoglucobrassicin, wheras the odor comes from the sulfur containing compounds allyl isothiocyanate, dimethyl trisulfide, dimethyl sulfide and methanethiol.

3.3.4 Brussels sprouts (*Brassica oleracea* Gemmifera Group)

Boiling and microwaving better help to prevent color losses in both fresh and frozen Brussels sprouts compared to steaming (Pellegrini *et al*, 2010). Frozen Brussels sprouts were also in general less affected by color change after having been cooked.

Carotenoids were retained in frozen Brussels sprouts independent of cooking method whereas boiling increased lutein in fresh Brussels sprouts but all other methods resulted in a significant decrease of β-carotene (Pellegrini *et al*, 2010).

In fresh Brussels sprouts the major glucosinolate was glucobrassicin followed by glucoiberin, which was also the major form present in frozen Brussels sprouts (Pellegrini *et al*, 2010). Steaming and microwaving resulted in increased glucosinolate levels in fresh Brussels sprouts, but all methods led to a decrease of glucosinolates in frozen Brussels sprouts.

As found in broccoli the ascorbic acid content is affected differently in fresh and frozen samples. Microwaving led to the largest losses in fresh Brussels sprouts where oven steaming was the best method to retain the levels. In frozen Brussels sprouts microwaving was the best way to retain the compound whereas boiling resulted in the greatest loss (Pellegrini *et al*, 2010).

Sinapic acid was the major phenolic compound in both fresh and frozen Brussels sprouts, and in the fresh samples naringenin was the major flavonoid whereas in frozen samples it was quercetin (Pellegrini *et al*, 2010). All cooking methods resulted in increased polyphenol levels for both fresh and frozen Brussels sprouts though oven steaming proved to be the worst method for fresh Brussels sprouts, resulting in significant decreases in both sinapic acid and flavonoids. Chlorogenic acid increased significantly independent of the treatment, which is probably due to its chemical interaction with the fiber being disrupted by the thermal treatments.

The Total Antioxidant Capacity (TAC) of fresh Brussels sprouts increased after boiling and basket steaming, probably due to the increase in carotenoids and quercetin (Pellegrini *et al*, 2010). In frozen Brussels sprouts a general decrease in TAC was noted due to the decreased amounts of ascorbic acid.

The glucosinolates sinigrin and progoitrin have been shown to be responsible for the bitter taste in cooked Brussels sprouts (van Doorn *et al*, 1998).

3.3.5 Cabbage (*Brassica oleracea* Capitata Group)

This report is going to bring up green, white and red cabbage in this section based on the information that is available on this subspecies.

The major glucosinolates in fresh green cabbage was found to be glucoiberin and sinigrin (Song *et al*, 2007) but it was also the one containing the least total amount of glucosinolates out of broccoli, Brussels sprouts, cauliflower and green cabbage both when fresh and after seven days storage in a refrigerator. Long time boiling (30 minutes) resulted in very significant decreases of the glucosinolate content in all subspecies tested but no other cooking method (0-20 minutes) resulted in any significant changes.

In red cabbage it was shown that some microwave treatments may increase the total glucosinolate content, something which might have been due to increasing extractability (Verkerk & Dekker, 2004). In the same study it was also shown that when microwaved with high power myrosinase completely lost its hydrolytic capacity, whereas it was somewhat retained even if microwaved for a long time at lower power levels. A good amount of myrosinase is essential for the health value of the vegetables since it is the breakdown products of the glucosinolates that have health promoting traits (Verkerk & Dekker, 2004). Regular cooking of red cabbage on the other hand has shown that indole glucosinolates seem more heat sensitive than aliphatic glucosinolates and will easier degrade (Oerlemans *et al*, 2006). All glucosinolates were degraded by temperatures of 100°C.

White cabbage has also been shown to have sinigrin as its major glucosinolate, as the authors Penas *et al* (2011) have found to be in line with previous studies. They were also able to establish other previous findings such as finding increased amounts of total glucosinolates where preharvest temperatures had been higher. This they have suspected was due to an increased production of precursors due to the higher temperatures. However there were differences found in which glucosinolates were found, which could be due to the different biosynthetic pathways not being equally temperature sensitive. Vitamin C was also shown to be higher when grown in higher temperatures, possibly as a defence mechanism against the higher temperatures and radiation. Total phenolic contents showed the reversed picture and was highest were growing temperatures were lower (Penas, 2011), probably due to the lower temperatures increasing the formation of reactive oxygen species (ROS) which the plant then needs to defend itself against (Klimov *et al*, 2008) .

3.3.6 Kale (*Brassica oleracea* Acephala Group)

The amino acid content in kale has also been found to decrease after cooking or freezing (Lisiewska, 2008). Kale has a higher concentration of flavonoids such as kaempferol and quercetin compared to many other vegetables (Hertog *et al*, 1992). Both of those flavonoids are believed to prevent cancer and other chronic diseases (Schmidt *et al*, 2010).

Neugart *et al* (2012) have shown that flavonols and especially the quercetin content clearly correspond to preharvest temperature changes in the variety sabellica. The quercetin content increased when plants were subjected to lower temperatures during their growth period, and decreased when subjected to higher temperatures. The isorhamnetin content appears to also be positively influenced by decreasing temperatures. The kaempferol content on the other hand decreased with decreasing temperature in the same variety (Schmidt *et al*, 2010). Higher temperatures therefore change the composition of flavonoids to contain more kaempferol rather than quercetin. The acquired forms of quercetin together have a higher antioxidant capacity than the acquired forms of kaempferol (Zietz *et al*, 2010), making the antioxidant capacity of kale lessen with higher temperature due to the change in composition. The increased quercetin-to-kaempferol ratio, which was seen in the experiments of Schmidt *et al* (2010), could have been triggered by the decreasing temperatures since lower temperatures have been correlated with higher numbers of ROS (Klimov *et al*, 2008). Neugart *et al* (2012) also established that the flavonol profile is influenced not only by temperature, but also by radiation and cultivar/variety. Several cultivars has shown no difference in total flavonoid concentration when temperatures decreased from 9,7 to 0,3°C (Schmidt *et al*, 2010), but Zietz *et al* (2010) has determined a significant increase in antioxidant activity when the temperature and radiation decreases down to a certain level (3,8°C and 179 µmol/m^{2}s) but when levels decreased further, no significant change was measured.

4. Discussion

Vegetables have a certain temperature range in which they can grow and retain their quality. If this range is exceeded it will lead to costs for or damages to the plant such as reduced growth, increased respiration rate and reduced carbon assimilation, the later which is essential for the photosynthesis (Guy, 1999). During the acclimation process the plant will start to produce higher amounts of already existing enzymes or even new forms and if the adaptation goes on for a long time these may become regular enzymes in the plant metabolism. However, it is not only the enzymatic activity and composition which is changed in order for the plant to keep up its cellular homeostasis and membrane structure and function may for example also be involved, as summarized by Guy (1999). This all means that vegetable plants which are subjected to preharvest temperatures outside their range are going to go through rather massive changes in their physiological processes when focus is shifted from growth to acclimation. The metabolism of the plants change due to the temperature change as the respiration and carbon assimilation rates are altered.

As Lurie (2006) stated, a lot of physiological postharvest processes such as senescence and ethylene production can be inhibited through proper heat treatment (adapted to the specific vegetable).This would make it possible to extend the shelf-life of the vegetables, which is probably one of the reason heat treatments are employed. The two processes are most probably not independent of one another since ethylene induces ripening and senescence when present. Thermal treatments executed with this purpose can still damage the vegetable if it is not carefully chosen and executed and since not all damages are external this can lead to growers not being aware that they have damaged a batch which may then be sent to customers even though the quality is reduced. If a treatment is not carried out appropriately this may also result in poor quality vegetables if their respiration and transpiration rates are not maintained or reduced since these factors can easily change together with the rest of the metabolism after harvest or after a treatment.

Concerning temperature processing it has been shown that fresh vegetables of the different subspecies of *B. oleracea* are better at retaining phytochemicals and antioxidant capacity after cooking than frozen ones (Pellegrini *et al*, 2010), which can be due to differences in the structural matrix of the cell walls. Frozen vegetables will on the other hand better preserve their color after cooking (Pellegrini *et al*, 2010), which is important in relation to the perceived quality. For the general nutritional quality the best cooking method is steaming for fresh vegetables of *B. oleracea* and for frozen samples it depends more on the vegetable and on which quality aspect is the most important to the consumer. Microwaving is best for keeping the color independent of whether fresh or frozen and also gives the best retention of glucosinolates, which is not to be taken lightly due to these vegetables being one of very few sources of it. Great ascorbic acid losses will be present no matter which cooking method is used. Only when too much emphasis is put on the ascorbic acid content is it therefore true that cooking reduces the nutritional quality, something which was previously often done.

All in all, temperature processing can help to retain and in some cases even enhance the nutritional values of vegetables of *B. oleracea* (Pellegrini *et al*, 2010) by increasing certain health promoting compunds, such as phenolic acids in Brussels sprouts and broccoli, carotenoids and quercitin in Brussels sprouts and glucosinolates in cauliflower. However, this is only the case if the right cooking method is used for the right product. Quality aspects such as color and a crispy texture can also be retained as long as the cooking period is not too long.

Pellegrini *et al* (2010) concluded that steaming may be the best cooking method for fresh broccoli, but not for frozen. This does not quite correlate with the knowledge which is brought to the public where steaming is generally seen as the best method for cooking broccoli no matter if frozen or fresh (Livestrong, 2011; Cleveland, 2010; Apotheken Umschau, 2012). This proves yet again that the need for research to be made more easily accessible and readable for the general public. If not there is a risk that conclusions are interpreted in the wrong way and the results of the studies will not be contributing to an actual improvement of the nutritional quality after processing.

As mentioned earlier in this report there is a lack of knowledge concerning how preharvest temperature stress affects *B. oleracea* and it seems reasonable to assume that this is true for most vegetables. The reason for this is probably the need for long term field studies, something that might be harder and more expensive to arrange than short term laboratory tests. It might also not be as big a concern as temperature processing, considering how vegetables of *B. oleracea* often are cooked before consumption and establishing the best cooking method for preserving or even enhancing the nutritional value and quality would therefore bring a big effect. However, climate change may change the current growing conditions and in worst case this could mean that the work which is currently being done to establish which genotypes are more nutritious will be for nothing if it later is discovered that they are weak to temperature stress during the growing period. It would also be interesting to see if temperature stress during different growth stages could improve the nutritional value of the vegetables. Therefore more research should be done in this field.

What can be stated based on the articles reviewed in this report is however that the effect of preharvest temperature stress is dependent on the genotype which is tested, and that the temperature affects different compounds differently, even in genotypes belonging to the same subspecies. No direct conclusions can therefore be done considering how preharvest temperature stress affects the nutritional quality of *B. oleracea* due to the lack of research done in the field and the great differences between genotypes. Concerning the other quality aspects preharvest temperature stress does not seem as important as postharvest temperature stress and temperature processing, since preharvest conditions generally do not affect the texture too much and color changes are mainly brought forth by radiation and postharvest handling such as not properly cooling the produce which shortens its shelf life and may cause a loss of greenness. Taste, however, can be affected preharvest but changes are often caused by mechanical damage which may cause the glucosinolates to mix with myrosinase which hydrolyses them to isothiocyanates that give a bitter taste to the vegetables (Mithen, 2000).

Not only Schmidt *et al* (2010) states that there is a clear difference in the nutritional quality between different genotypes (cultivars) of the subspecies, but several authors claim the same thing (Singh *et al*, 2007; Kurilich *et al*, 1999; Oerlemans, 2006). This indicates a need to make consumers more aware of which cultivars they are buying and should possibly be suggested to the supermarkets or to the media in order to create consumer interest.

There is a clear confusion concerning the terminology used when discussing health promoting and quality reducing features and this does increase the risk of misinterpreting the articles read, since some authors do not define what they are talking about when they for example mention a vitamin. There are often several different forms of a vitamin such as vitamin C, but in this report the only vitamin C compound which is commonly discussed is ascorbic acid or ascorbate. This is a possible source of error in the report which the reader should be aware of. It should also be noted that many of the biosynthetic pathways discussed in this report interact with pathways of other substances through different intermediaries. If a specific intermediary would be involved in several pathways for different health promoting or quality reducing compounds this would make it a key compound. This would mean that many pathways are dependent on one single intermediary which may or may not be replaceable, if it was to be heat sensitive for example. There is therefore a need to look at the whole biosynthetic pathway of a compound when assessing how it is affected by temperature or other abiotic factors. Not only should the effect on the end product be studied, but also the effect on the intermediaries in its pathway in order to foresee effects on other compounds as well through the connection of the intermediaries.

5. Conclusions

The conclusions which can be made based on this report are:

- Much research is still needed in order to better grasp how temperature affects different aspects of a plant life.
- Vegetables go through major physiological changes concerning for example hormone expression, metabolism, enzyme composition and membrane structure when put through temperature stress.
- The nutrional value of *B. oleracea* subspecies varies with the different genotypes and this is something which needs to be taken seriously if a vegetable is to be promoted as "healthy".
- Temperature processing such as cooking can increase certain health promoting compounds such as phenolic acids in broccoli and Brussels sprouts, glucosinolates in cauliflower as well as carotenoids and flavonoids in Brussels sprouts. This means that the nutritional value of *B. oleracea* can be enhanced if the right cooking method is used. For fresh vegetables this can be done through steaming whereas it for frozen vegetables is more dependent on which species is being cooked and which quality aspects the consumer finds most important. Preharvest treatments do not show as clear a pattern but there are indications that some health promoting compounds such as ascorbic acid and glucosinolates are produced more in slightly higher temperatures.
- Temperature processing like cooking can reduce the perceived quality of the vegetable as it loses greenness, color saturation and may also easily get a too soft texture and start smelling from the sulfur compounds and taste bitter due to the breakdown of glucosinolates. However, this breakdown also gives rise to several highly interesting compounds believed to play a role in the prevention of degenerative diseases. Therefore it can be said that heat treatments do reduce the quality which we can sense, but it may induce the invisible aspects of quality. Not much is known about how preharvest treatments affect the (sensory) quality.

6. References

Abou-Hussein, S.D. (2012) Climate change and its impact on the productivity and quality of vegetable crops (review article). *Journal of Applied Sciences Research* 8 (8), 4359-4383.

Ames, B.N.; Shigenaga, M.K.; Hagen, T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America* 90, 7915-7922

Apotheken Umschau (2012-09-20). Wie Sie Gemüse richtig zubereiten. http://www.apotheken-umschau.de/Ernaehrung/Wie-Sie-Gemuese-richtig-zubereiten-115479.html [2013-05-09]

Cartea, M.E.; Velasco, P.; Obregon, S.; Padilla, G.; De Haro, A. (2008) Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. *Phytochemistry* 69, 403–410.

Cleveland (2010-08-03). *Raw veggies pack a punch, but cooking can unlock key benefits.* http://www.cleveland.com/healthfit/index.ssf/2010/08/raw_veggies_can_pack_a_punch_b.html [2013-05-09]

Cornell University (2013-01-16). *Glucosinolates (goitrogenic glycosides)* http://www.ansci.cornell.edu/plants/toxicagents/glucosin.html [2013-05-09]

Dixon, G.R. (2007) Crop production science in horticulture 14: Vegetable Brassicas and related crucifers. King's Lynn, UK: CAB International

Engel, E.; Baty, C.; le Corre, D.; Souchon, I.; Martin, N. (2002) Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *Journal of Agricultural and Food Chemistry* 50, 6459-6467.

Evangelou, A.; Kalpouzos, G.; Karkabounas, S.; Liasko, R.; Nonni, A.; Stefanou, D.; Kallistratos, G. (1997) Dose-related preventive and therapeutic effects of antioxidants-anticarcinogens on experimentally induced malignant tumors in Wistar rats. *Cancer Letters* 115, 105-111.

FAO: Food and Agriculture Organization of the United Nations (2013-01-16). *FAOSTAT - Production – Crops, Value of Agricultural Production.* http://faostat.fao.org/site/567/default.aspx#ancor [2013-05-09]

Gebczynski, P. & Lisiewska, Z. (2006) Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. *Innovative Food Science & Emerging Technologies* 7, 239–245.

Griffiths, D.W.; Birch, A.N.E.; Hillman, J.R. (1998) Antinutritional compounds in the Brassicaceae: analysis, biosynthesis, chemistry and dietary effects. *Journal of Horticultural Science and Biotechnology* 73, 1–18.

Guy, C. (1999) Molecular responses of plants to cold shock and cold acclimation. *Journal of Molecular Microbiology and Biotechnology* 1 (2), 231-242.

Hertog, M.G.L.; Hollman, P.C.H.; Katan, M.B. (1992) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry* 40, 2379-2383.

Klimov, S.V.; Burakhanova, E.A.; Dubinina, I.M.; Alieva, G.P.; Sal'nikova, E.B.; Olenichenko, N.A.; Zagoskina, N.V.; Trunova, T.I. (2008) Suppression of the source activity affects carbon distribution and frost hardiness of vegetating winter wheat plants. *Russian Journal of Plant Physiology* 55 (3), 308-314.

Jahangir M.; Bayoumi Abdel-Farid I.; Kyong Kim, H.; Hae Choi, Y.; Verpoorte, R. (2009) Healthy and unhealthy plants: The effect of stress on the metabolism of Brassicaceae. *Environmental and Experimental Botany* 67, 23-33.

Kotak, S.; Larkindale, J.; Lee, U.; von Koskull-Döring, P.; Vierling, E.; Scharf, K.-D. (2007) Complexity of the heat stress response in plants. *Current Opinion in Plant Biology* 10, 310-316.

Kurilich, A.C.; Tsau, G.J.; Brown, A.; Howard, L.; Klein, B.P.; Jeffery, E.H.; Kushad, M.; Wallig, M.A.; Juvik, J.A. (1999) Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry* 47, 1576-1581

Lisiewska, Z.; Kmiecik, W.; Korus, A. (2008) The amino acid composition of kale (*Brassica oleracea* L. var. acephala), fresh and after culinary and technological processing. *Food Chemistry* 108, 642–648.

Liu, R.H. (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition* 78, 517S-520S.

Livestrong (2011-09-03). *Healthiest way to cook frozen broccoli without losing nutrients*. http://www.livestrong.com/article/534770-healthiest-way-to-cook-frozen-broccoli-without-losing-nutrients/ [2013-05-09]

Livsmedelsverket (National Food Agency) (2012-04-20). *Frukt och grönt*. http://www.slv.se/sv/grupp1/Mat-och-naring/Kostrad/Vuxna/Frukt-och-gront-/ [2013-04-18]

Lurie, S. (2006) The effects of high temperature treatment on quality of fruits and vegetables. *Acta Horticulturae* 712 I , 165-173

Mithen, R.F.; Dekker, M.; Verkerk, R.; Rabot, S.; Johnson, I.T. (2000) The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture* 80, 967-984.

Moreno, D.A.; Carvajal, M.; Lopez-Berenguer, C.; Garcia-Viguera, C. (2006) Chemical and biological characterisation of nutraceutical compounds of broccoli. *Journal of Pharmaceutical and Biomedical Analysis* 41, 1508–1522.

Neugart, S.; Kläring, H.-P.; Zietz, M.; Schreiner, M.; Rohn, S.; Kroh, L.W.; Krumbein, A. (2012) The effect of temperature and radiation on flavonol aglycones and flavonol glycosides of kale (*Brassica oleracea* var. sabellica). Food Chemistry 133, 1456-1465.

Oerlemans, K.; Barrett, D.M.; Bosch Suades, C.; Verkerk, R.; Dekker, M. (2006) Thermal degradation of glucosinolates in red cabbage. *Food Chemistry* 95, 19-29.

Pellegrini, N.; Chiavaro, E.; Gardana, C.; Mazzeo, T.; Contino, D.; Gallo, M.; Riso, P.; Fogliano, V.; Porrini, M. (2010). Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen Brassica vegetables. *Journal of Agricultural and Food Chemistry* 58, 4310-4321.

Penas, E.; Frias, J.; Martinez-Villaluenga, C.; Vidal-Valverde, C. (2011) Bioactive compounds, myrosinase activity, and antioxidant capacity of white cabbages grown in different locations of Spain. *Journal of Agricultural and Food Chemistry* 59, 3772-3779.

Podsędek, A. (2007) Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. *LWT – Food Science and Technology* 40, 1–11.

Schmidt, S.; Zietz, M.; Schreiner, M.; Rohn, S.; Kroh, L.W.; Krumbein, A. (2010) Genotypic and climatic influences on the concentration and compostion of flavonoids in kale (*Brassica oleracea* var. sabellica). *Food Chemistry* 119, 1293-1299.

Schonhof, I.; Klaring, H.P.; Krumbein, A.; Claussen, W.; Schreiner, M. (2007a) Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. *Agriculture, Ecosystems & Environment* 119, 103–111.

Schonhof, I.; Blankenburg, D.; Müller, S.; Krumbein, A. (2007b) Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. *Journal of Plant Nutrition and Soil Science* 170, 1-8

Sies, H. (1997) Oxidative stress: Oxidants and antioxidants. *Experimental Physiology* 82, 291-295.

Sikora, E.; Cieslik, E.; Leszczynska, T.; Filipiak-Florkiewicz, A.; Pisulewski, P.M. (2008) The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food Chemistry* 107, 55–59.

Singh, J.; Upadhyay, A.K.; Prasad, K.; Bahadur, A.; Rai, M. (2007) Variability of carotenes, vitamin C, E and phenolics in *Brassica* vegetables. *Journal of Food Composition and Analysis* 20, 106-112.

Song, L.; Thornally, P.J. (2007) Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and Chemical Toxicology* 45, 216-224.

Temple, N.J. (2000) Antioxidants and disease: More questions than answers. *Nutrition Research* 20 (3), 449-459.

van Doorn, H.E.; van der Kruk, G.C.; van Holst, G.-J.; Raaijmakers-Ruijs, N.C.M.E.; Postma, E.; Groeneweg, B.; Jongen, W.H.F. (1998) The glucosinolates sinigrin and progoitrin are important determinants for taste preference and bitterness of Brussels sprouts. *Journal of the Science of Food and Agriculture* 78, 30-38

Verkerk, R. & Dekker, M. (2004) Glucosinolates and myrosinase activity in red cabbage (*Brassica oleracea* L. var Capitata f. rubra DC.) after various microwave treatments. *Journal of Agricultural and Food Chemistry* 52, 7318-7323.

WH Foods (2001) What are goitrogens and in which foods are they found? http://www.whfoods.com/genpage.php?tname=george&dbid=47 [2013-05-09]

Zietz, M.; Weckmüller, A.; Schmidt, S.; Rohn, S.; Schreiner, M.; Krumbein, A.; Kroh, L.W. (2010) Genotypic and climatic influence on the antioxidant activity of flavonoids in kale (*Brassica oleracea* var. sabellica). *Journal of Agricultural and Food Chemistry* 58, 2123-2130.