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EFFECT OF THERMAL TREATMENT ON CAROTENOIDS IN CAULIFLOWER
EFFETTO DEL TRATTAMENTO TERMICO SUI CAROTENOIDI NEL CAVOLFIORE

TIPO TESI: (sperimentale)

Studente:
Benedetta Fanesi

Relatore:
PROF. DEBORAH PACETTI

Correlatore:
DOTT. ALESSANDRA GIARDINIERI

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Riassunto

Il cavolfiore (*Brassica oleracea* var. *botrytis*) è uno dei vegetali più ricchi di componenti bioattivi, tra cui i carotenoidi. I carotenoidi sono pigmenti con colorazioni che vanno dal giallo al rosso passando per l'arancione. Essi si dividono in due gruppi: i "caroteni", composti da una catena idrocarburica e, le "xantofille" costituite non solo da atomi di carbonio e idrogeno, ma anche ossigeno (risultando più polari rispetto i caroteni). Essi vengono sintetizzati all'interno dei cloroplasti e cromoplasti di organismi fotosintetici (tra cui frutti e vegetali) con lo scopo di catturare la luce e soprattutto di deattivare i radicali liberi, infatti sono delle sostanze "antiossidanti". D'altra parte, i carotenoidi non sono sintetizzati ex novo dagli animali (compreso l'uomo), per cui devono essere introdotti mediante la dieta. Nell'uomo svolgono però importanti funzioni oltre all'attività antiossidante, come: prevenzione di malattie cardiovascolari, proprietà anticancerogene e antiinfiammatorie e sono funzionali per la vista.

Per queste ragioni, lo scopo di questa ricerca è stato quello di indagare quale fosse l'effetto del trattamento termico su questi composti presenti nel cavolfiore arancione (varietà "Cheddar") e viola (varietà "Graffiti")

I campioni dopo essere stati accuratamente tagliati ed omogenizzati, sono stati sottoposti a tre diversi metodi di cottura con differenti tempi di trattamento: bollitura (10 e 25 minuti), cottura al forno con vapore e cottura sous-vide (entrambe a 10, 25 e 40 minuti). Successivamente, sono stati liofilizzati e confezionati sottovuoto.

L'estrazione è stata effettuata seguendo la metodica ripresa da Biswas, Sahoo, and Chatli (2011). Per l'analisi è stato utilizzato un sistema UPLC e un rilevatore PDA a 450 nm. I carotenoidi sono stati identificati comparando i tempi di ritenzione con quelli degli standard puri, mentre la quantificazione è stata calcolata costruendo rette di calibrazione. I dati sono stati successivamente analizzati con il sistema statistico dell'ANOVA.

Nei cavolfiori arancioni e viola sono stati identificati i seguenti carotenoidi: luteina (xantofilla), β-carotene e α-carotene (caroteni). Comparando i risultati ottenuti da ciascun trattamento termico con il rispettivo crudo, si è visto che la cottura incrementa il contenuto di tutti i carotenoidi. La ragione di questo fenomeno è stata attribuita a: la rottura delle strutture cellulari e dei complessi proteina-carotenoide, rendendoli maggiormente biodisponibili e, inoltre l'inattivazione degli enzimi che degradano tali composti.

Il nostro studio ha messo in luce come la bollitura sia la migliore tecnica di cottura: la bollitura per 25 minuti riesce ad estrarre in maniera più efficiente i carotenoidi dalla struttura vegetale e nello stesso tempo la temperatura di 100°C non comporta la distruzione dei carotenoidi estratti.

Le cotture al forno con vapore e sous-vide non hanno mostrato valori statisticamente significativi né tra i due diversi metodi né tra tempi di cottura diversi in ciascun metodo, ma comunque risultati migliori rispetto al crudo.

Per concludere, il rapporto β-carotene/luteina è stato calcolato ed analizzato statisticamente per vedere se il comportamento dei due carotenoidi (il primo apolare, il secondo polare) variasse nei diversi trattamenti termici. Il risultato è stato negativo, dimostrando che entrambi i carotenoidi hanno un comportamento simile.

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Acronyms and abbreviations

AMD	Age related macular degeneration
CA	Orange Cauliflower
CV	Purple Cauliflower
DNA	Deoxyribo Nucleic Acid
FDA	Food and Drug Administration
HDL	High density lipoproteins
HHP	High Hydrostatic pressure
LDL	Low density lipoproteins
PDA	Photo Diode Array
UPLC	Ultra Pressure Liquid Chromatography
UV	Ultra-violet

1. Introduction

1.1 General informations

For the first time, in 1831, carotenoids were found in carrots by Wackenroder, a German pharmacist. He was searching for an anthelminthic, a medication for worms, in vegetables. He obtained the pigment as small ruby-red flakes and then, he dissolved it in fats like butter and it imparted "a beautiful yellow colour". Consequently, it took the common name of "carotene"(Sourkes 2009).

Carotenoids appeared very early in the history of life on Earth. They were present in one of the first inhabitants of our planet: cyanobacteria, the oldest known oxygenic photosynthetic organism.

Nowadays, we know about 700 carotenoids and we found them: in plants, animals, bacteria, fungi etc (Melèndez-Martìnez 2016). Carotenoids can be biosynthesized only by algae, plants and photosynthetic bacteria, not by animal organism. Anyway, carotenoids are found in animals since they are introduced by the diet.

On the contrary, carotenoids are synthesized in the chloroplasts and chromoplasts of all photosynthetic organisms and they are responsible for their yellow, orange and red colours (Lachman et al. 2016).

Carotenoids are antioxidants and they are considered "phytochemicals" or bioactive compounds produced by plants. They don't have nutritive functions as vitamins and minerals, but they are important for human health and to prevent many diseases.

1.2 Chemical structure

Carotenoids are divided in two groups: "carotenes", that have just carbon and hydrogen atoms and, "xanthophylls", that have also oxygen atoms contained in different functional groups like hydroxy, carbonyl, carboxylic or epoxide groups. Some examples of carotenes are: β-carotene, α-carotene, lycopene, while xanthophylls are lutein, zeaxanthin, violaxanthin etc.

The basic carotenoid structure consists of isoprene units linked covalently in either a head-to-tail or tail-to-tail. A typical carotenoid structure has 40 carbon atoms (Figure 1), (Saini, Nile, and Park 2015) although they can have also more or fewer atoms (Melèndez-Martìnez 2016).

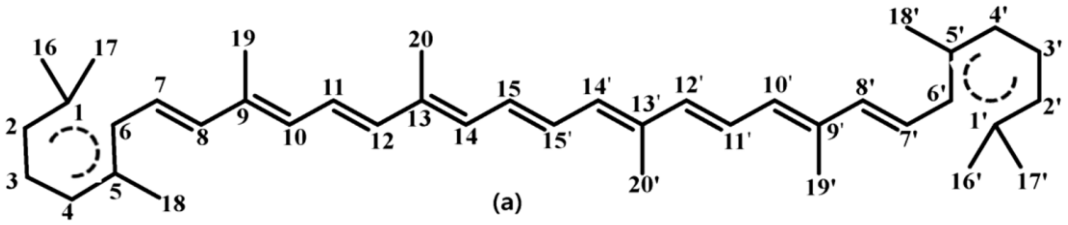


Figure 1. A typical carotenoid's structure.

Thanks to the system of double bonds chemical structure results the property of carotenoids to appear coloured because carotenoids are able to interact with the light reflecting or releasing energy to different lengths. This interaction is responsible for colours. Thus, more double bonds there are, more intense colour is, for example: phytofluene, with 5 double bonds, is the less coloured; lycopene, with 11, is the most coloured; while ß-carotene is not as coloured as lycopene because the first one is cycled and the second one not. Really, colour intensity depends on the presence of other particular groups such as =N, =O, =S, -OH (Paolo and Aldo 2004), but also on the concentration, the physical state, technological treatments suffered and the presence of other pigments like chlorophyll (Mignogna 2010).

Generally, these pigments have colours from red to yellow by way of orange and pink, even if they can seem other colours when they form complexes with other molecules like fatty acids or proteins.

The main absorption of carotenoids falls within the 400-500 nm wavelengths region, even if they show not just a single absorption band but three more or less peaks (Britton, Liaaen-Jensen, and Pfander 2004).

1.2.1 Esters

Carotenoids can be also free or esterified with other molecules like long-chain or medium-chain fatty acid or glycosides, so they change their lipophilicity and hydrophilicity respectively. Other carotenoids can also form complexes with proteins leading to increase stability and change their colours, and this is typical in invertebrates, fish, shrimps and molluscs (Melèndez-Martìnez 2016).

In the chromoplast of plants most prevalent carotenoids are the xanthophylls, which are esterifies with fatty acids. Esterification facilitates the accumulation of xanthophylls within the chromoplast (R. K. Saini, 2015).

1.2.2 Isomers

Given that carotenoids have double bonds in the structure, different geometrical isomers (cis/trans or better Z/E) can be found. Z isomers have an angular shape, while E isomers have a linear shape. In nature, they are present in all-trans (all-E) isomeric form. However, they can

undergo mono or poly isomerization by light, thermal energy and chemical reactions to their cis-isomeric forms (Figure 2), (Tanumihardjo 2013).

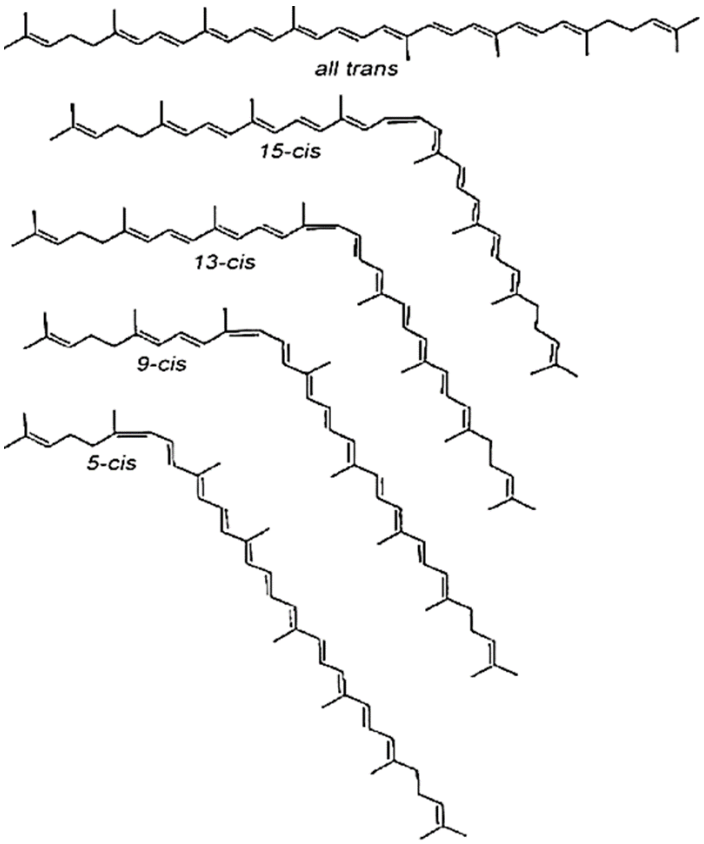


Figure 2. Type of isomers

Some carotenoids can also have chiral centre, so they form different optical isomers, like zeaxanthin. It is important to note that they adopt a specific preferred conformation of low energy (Melèndez-Martìnez 2016).

1.2.3 Apocarotenoids

Apocarotenoids are another class of carotenoids, derived from carotenoids by oxidative cleavage, catalysed by family of carotenoid cleavage dioxygenases (CCDs). A great diversity of apocarotenoids was found in plants, animals and microorganisms. Biologically and commercially important apocarotenoids include vitamin A, retinoids, retinol and retinoic acid, abscisic acid, bixin and aromatic volatile aroma compounds ß- and α- ionone (Figure 3).

CCDs play fundamental roles in carotenoids degradation in plants, by negatively regulating the carotenoid content. For example, if CCD' genes are destroyed, the content of carotenoids increases (such as in potato plants) or can also change flower colour from white to yellow (such as in *Brassica* species). On the other hand, CCDs' products play an important role in

attraction of pollinators, herbivore deterrence and defence against pathogen. Yet they are important to flavour, fragrances, signalling and many aspects of plant growth development (Saini, Nile, and Park 2015).

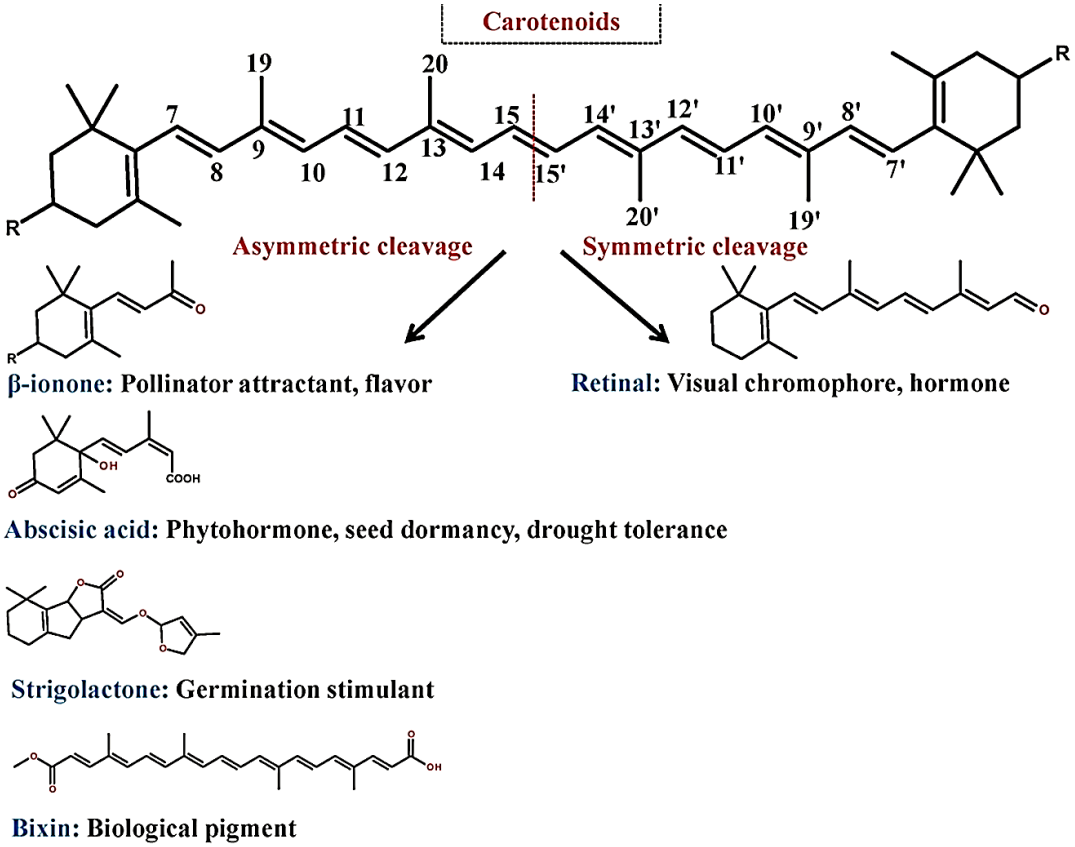


Figure 3. Apocarotenoids

1.3 Physical and chemical properties

All carotenoids are lipophilic compounds and so they are soluble in oils and organic solvents. Yet, they can be isomerized by heat, acid or light. Many carotenoids exhibit spectral shift after reaction with various reagents. During extraction procedures hexane-acetone mixtures are the most used, even if special solvents and treatments are sometimes needed to achieve satisfactory separation.

In addition, carotenoids can be also oxidized due to the large number of conjugated double bonds. Such reactions cause colour loss of carotenoids in foods and are the major degradation mechanism of concern (Fennema 1996).

From the chemical point of view, thanks to their particular structure, they can perform many functions. In plants, they contribute to the harvesting of light, at different wavelengths compared to chlorophylls. They deactivate free radicals, or rather single oxygen atoms that can damage cells by reacting with other molecules.

Among living beings, carotenoids are important for the communication. The colour of flowers attracts the pollinators promoting the propagation of species. Animals use colours for species recognition, warning, mimicry, crypsis, sexual signalling or social status reporting.

In humans, they are important for vision and they have antioxidant activity, cancer-fighting properties, anti-inflammatory and immune system benefits and prevention of cardiovascular disease, too.

Moreover, it is supposed that carotenoids can be an important role in fertility and reproduction because high levels of carotenoids were found in egg yolk (Szalay 2015; Melèndez-Martìnez 2016).

1.4 Principal carotenoids

As it has just said before, carotenoids are divided in "carotenes" and "xanthophyll". Really, from nutritional point of view, it is also possible to separate them in: provitamin A and not-provitamin A. α-carotene, ß-carotene and ß-cryptoxanthin are provitamin A carotenoids; lutein, zeaxanthin and lycopene are not-provitamin A. These six carotenoids are the most studied and, even if in the second group there are not provitamins, they always have a great interest because they have a major antioxidant activity (Szalay 2015).

1.4.1 ß-carotene

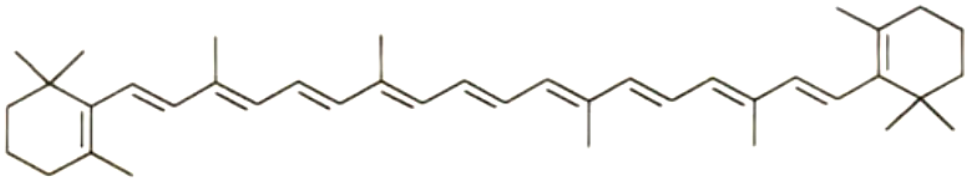


Figure 4. β-carotene

ß-carotene is a yellow pigment and has formula $C_{40}H_{56}$ (Figure 4) (Britton, Liaaen-Jensen, and Pfander 2004).

It is represented the molecule with all-trans bonds, but it exists also cis isomers for example: 9-cis, 13-cis, 15-cis and 11,15-cis (Fennema 1996).

It has two ß-ionone rings at the two ends and it is the most powerful when it comes to turning into vitamin A; twice as much ß-carotene becomes vitamin A than does α-carotene or ß-cryptoxanthin (Szalay 2015)as it is shown in Table 1 (Saini, Nile, and Park 2015)

Table 1. Relative provitamin A activity of common food carotenoids.

Carotenoid	% provitamin A activity
All-E-ß-carotene	100
All-E-ß-cryptoxanthin	57
13-Z-ß-carotene	53
All-E-α-carotene	53
15-E-ß-cryptoxanthin	42
9-E-ß-carotene	38
9-Z-ß-cryptoxanthin	27
ß-carotee-5,6-epoxide	21
13-Z-α-carotene	16
9-cis-α-carotene	13

ß-carotene is the most abundant natural carotene, with a wide distribution in plants, algae, fungi, bacteria and animals. Within vegetables and fruits groups, those with the major content of its are, for example, carrots, pumpkins, green leaves, melons, mangoes and apricots (Paolo and Aldo 2004).

It seems to be capable of both positive and negative effects, especially for smokers taking it as a supplement. In these people, it can increase their risk of lung cancer. On the other hand, ß-carotene may help protect against sunburn, help lower the risk of metabolic syndrome, that consists into high blood pressure, high blood sugar, abnormal cholesterol levels and excess fat around waist. The men with the most ß-carotene intake had the lowest risk of metabolic syndrome, as well as reduced waist circumference. Scientists suspect this is the result of ß-carotene's antioxidant activity (Szalay 2015).

1.4.2 α-carotene

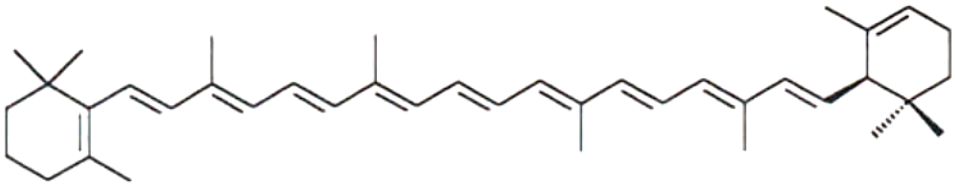


Figure 5. α-carotene

α-carotene is a yellow pigment and has formula $C_{40}H_{56}$, like ß-carotene (Figure 5) (Britton, Liaaen-Jensen, and Pfander 2004). It produces half the vitamin A that ß-carotene does. It is widely distributed in similar foods to ß-carotene such as pumpkin, carrots, tomatoes and peas, but in lower concentration. In addition to provitamin A function, it also has some potential longevity benefits. Actually, it has seen the correlation between high levels of α-carotene and

a lower risk of death from diabetes, cancer, cardiovascular disease and all other illness causes (Szalay 2015).

1.4.3 Lycopene

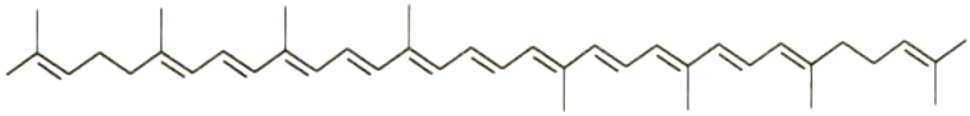


Figure 6. Lycopene

Lycopene (Figure 6) (Britton, Liaaen-Jensen, and Pfander 2004)has formula $C_{40}H_{56}$ and not cycle, so it is a bright red pigment. It is present especially in tomatoes, but also in watermelons, grapefruits, papaya, red cabbage, asparagus and red bell peppers. The unique shape of lycopene makes it to be the most effective at deactivating singlet oxygen and other free radicals. Many studies demonstrate benefits of lycopene, for example: it reduces prostate and lung cancer, osteoporosis and stroke risks (Szalay 2015).

1.4.4 ß-cryptoxanthin

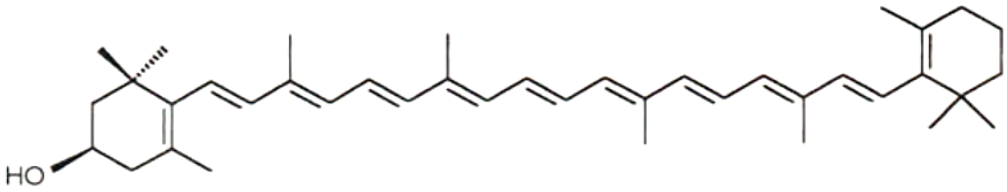


Figure 7. β-cryptoxanthin

ß-cryptoxanthin (Figure 7) has formula $C_{40}H_{56}O$, is a yellow xanthophyll. It usually occurs in small amounts in many plants and fruits such as corn, oranges and citrus fruit. Commonly, it is present as esters with fatty acids.

It is also provitamin A, even if it produces half as much as ß-carotene. Many studies have demonstrated that it is effective in preventing cancer risk with lutein and zeaxanthin. Moreover ß-cryptoxanthin may be helpful in reducing the risk of inflammatory rheumatoid arthritis (Szalay 2015).

1.4.5 Lutein

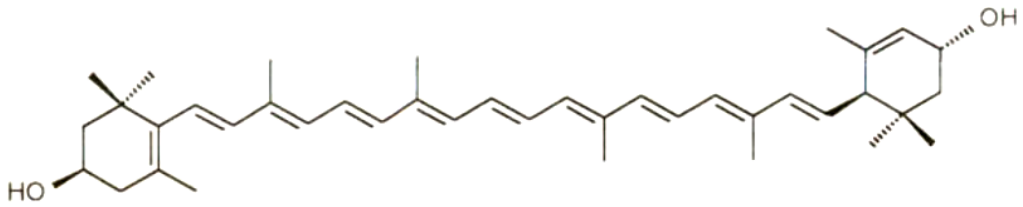


Figure 8. Lutein

Lutein (Figure 8) has formula $C_{40}H_{56}O_2$. It is a yellow xanthophyll. This pigment is typical of green leaves, fruits and flowers such as marigold and sunflowers (Britton, Liaaen-Jensen, and Pfander 2004).

Lutein is known to prevent the formation of atherosclerosis, which is composed of plaques that restrict blood flow to the heart muscle. When lutein is in the blood, it can have an antioxidant effect on cholesterol, thereby preventing cholesterol from building up in the arteries and clogging them.

Furthermore, lutein is usually associated to zeaxanthin, because they are the only two xanthophylls found in human retina at the macula lutea, which is responsible for central vision and protects the retina from blue light, which may cause damage the retina. Actually, a study demonstrated that people who have consumed in adequate quantities on a daily basis, they reduced the risk of age-related macular degeneration (AMD) and the incidence of cataract and light sensitivity (Szalay 2015).

1.4.6 Zeaxanthin

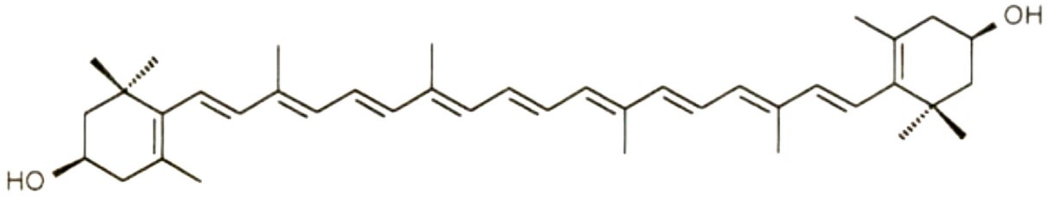


Figure 9. Zeaxanthin

Zeaxanthin (Figure 9) has formula $C_{40}H_{56}O_2$ and is another yellow xanthophyll. It is the major carotenoid from yellow maize (*Zea mays*) from which it takes its name. Yet, it also occurs in saffron.

Zeaxanthin is a chiral carotenoid, so exists another form: meso-zeaxanthin, even if it does not have optical activity. The latter is isolated from some shrimps, fish and it is the main form of zeaxanthin in the macula of the human eye.

Generally, zeaxanthin is accompanied by larger amounts of lutein in plants and algae, as it has just said beyond.

1.5 Food sources

Carotenoids are very widespread in nature. They are present everywhere in fruits, vegetables and animals. Among the last group, the most common carotenoids are echinenone, astaxanthin, canthaxanthin, lutein and zeaxanthin. They are very important especially for the relationship between species. Many studies showed that animals cannot biosynthesize these pigments, but they have to introduce them through diet and then they can converted them to produce other colour, as it happens for feathers of birds (Weaver et al. 2018).

Yet, the main food sources of carotenoids remain fruits and vegetables, as Table 2 shows. Most of the time the type of carotenoids can be predicted by their colour. Generally, in yellow-orange fruits and vegetables like apricots, peaches, mangoes, papayas, carrots and pumpkins there is β-carotene, α-carotene, β-cryptoxanthin, lutein and zeaxanthin. Tomatoes are very rich in lycopene and to a lesser extent also watermelons and pink grapefruits. In green leafy vegetables, lutein, β-carotene, violaxanthin and neoxanthin are predominant (Zakynthinos and Varzakas 2016; Saini, Nile, and Park 2015).

Table 2. Content of major carotenoids in selected fruits and vegetables. (µg/g fresh weight)

FRUIT	**Lutein**	**Zeaxanthin**	**B-cryptoxanthin**	**A-carotene**	**B-carotene**	**lycopene**	**Total carotenoids**
Grapes	0.47	-	-	-	0.23	-	-
Mango	31.7	1.5	-	-	-	-	-
Papaya	237	14.1	-	-	-	-	-
Peach	-	1.1	1.6	-	9.3	-	12.0
Pineapple	-	-	-	-	9.9	-	-
Watermelon	172	-	-	-	-	7.0	185
VEGETABLE							
Broccoli	28.05	-	0.15	-	11.38	-	46.46
Carrot	1.5	-	-	45.0	53.6	-	-
Cauliflower (white)	0.28	-	0.15	-	0.22	-	0.746
Corn	13.1	6.2	1.7	-	-	-	-
Lettuce	13.5	-	-	-	14.9	-	-
Pumpkin	-	-	-	39.9	172.2	-	236.1
Spinach	775.8	15.1	-	-	365.3	-	2386.2

Furthermore, many studies demonstrate the qualitative and quantitative composition of carotenoids varies with multiple factors such as: cultivar, variety, maturity at harvest, climate, farming practice and post-harvest processing and storage (Walsh, Bartlett, and Eperjesi 2015).

1.6 Bioavailability and bioaccessibility

Bioavailability is the portion of the digested nutrients or phytochemicals that are absorbed and metabolized by normal pathways and is normally measured by in vivo methods. Meanwhile, bioaccessibility is defined as the amount of a component that is released from the food matrix into the gastrointestinal tract and available for absorption.

In vitro models are commonly used to measure it because they allow to simulate gastrointestinal digestion and provide important information for both researcher and food industry (Barba et al. 2017). Actually food processing is one of the main determinants on bioavailability because it can have a positive or negative impact by increasing or decreasing bioaccessibility of bioactive compounds (Cilla et al. 2018).

Food processing and cooking cause the mechanical breakdown of the tissue releasing the carotenoids and improving their absorption. They solubilize in micelles that are formed by dietary fat and bile acids, then they are taken up by epithelial cells of intestine tract by passive diffusion and secreted to lymphatic system as chylomicron. In this way carotenoids arrived to tissues and there can be accumulated and metabolized (Zakynthinos and Varzakas 2016; Tanumihardjo 2013).

Carotenoids are always transported thanks to lipoproteins in the blood serum even if carotenes circulate with low-density lipoproteins (LDL), while xanthophylls with high-density lipoproteins (HDL), because the last are more polar than the first. Liver and adipose tissue are the main sites of carotenoid deposition in humans.

The bioavailability depends on factors such as the degree of processing of foods, the level and type of dietary fat and the presence of other carotenoids in food. Actually, dietary fat enhances the solubilization of them into lipid globules and their absorption. On the other hand, dietary fibre decrease the bioavailability by disrupting micelle formation (Food Standards Australia New Zealand 2017).

1.7 Benefits

Carotenoids with other phytochemicals such as phenolics, alkaloids, nitrogen-containing compounds, organosulfur compounds and phytosterols play important roles in the prevention of chronic diseases. This suggests that changes in dietary patterns, lifestyle and increasing the consumption of fruits and vegetables are effective strategy for reducing the incidence of many illnesses. Many epidemiological studies have examined the role of phytochemicals and increased dietary intake of fruits and vegetables. It is demonstrated, for example, the consumption of flavonoids in humans is inversely correlated with mortality from coronary

heart disease and myocardial infraction. This because, is associated to decrease of the LDL cholesterol and plasma total cholesterol concentrations (Liu 2013).

Focused on carotenoids, they explicate important functions as showed in Figure 10 (Tanumihardjo 2013).

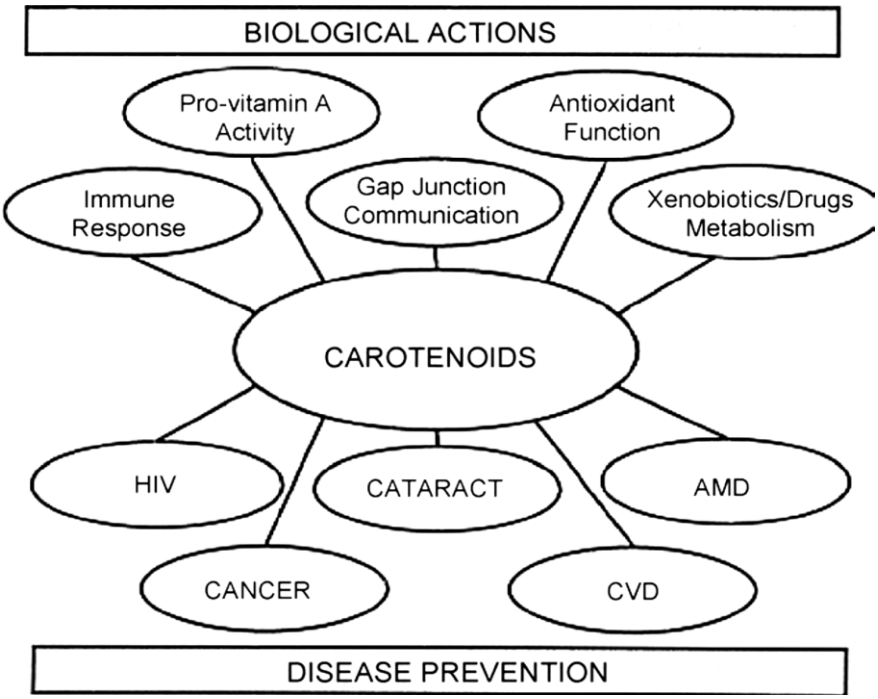


Figure 10. Biological actions of carotenoids and disease prevention correlated.

Carotenoids such as α- and β-carotene and β-cryptoxanthin have antioxidant activity and can be converted to vitamin A, related in the development and disease prevention. β-carotene also reduces the risk of lung cancer and its antioxidant activity is stronger if it is consumed with other phytochemicals. Yet, higher intake of β- carotene produces pro-oxidant effect (Tanumihardjo 2013).

In human plasma, the predominant carotenoid is lycopene, where half of the total is present as cis isomer, even if in nature all trans isomeric form is the most abundant.

A case-control study on Vietnamese men with a higher intake of lycopene, tomatoes, and carrots pointed out a lower risk of prostate cancer. Lycopene can prevent DNA by scavenging free radicals, modulating the gene expression and slowing cancer cell growth. In addition, it hampers the progression of prostate cancer via apoptosis induction and angiogenesis suppression (Van Hoang et al. 2018).

Another case-control study examined associations between dietary intake of lutein and zeaxanthin and the risk of Age-related Macular Degeneration (AMD). AMD is one of the leading causes of blindness in older adults. It increases with advancing age and appears to be

more likely in men than women. Lutein and zeaxanthin along with their isomer meso-zeaxanthin are the major carotenoids accumulated in the retina of the eye and form the retinal macular pigment. They have several functions in improving visual performance and protecting against the damaging effects of light (Eisenhauer et al. 2017).

1.8 Heat treatments

It is established that fruits and vegetables are rich on bioactive compounds, which are great beneficial for human health. Sometimes they are consumed fresh, but in most cases, cooking is essential to obtaining more digestible, safe and convenience food products (Paciulli et al. 2018).

Food processing can have negative or positive effects on carotenoids. The loss of carotenoids during processing and storage of foods can occur through isomerization and enzymatic or non-enzymatic oxidation. Yet, heat treatment can increase the bioavailability of them as it inactivates enzymes and breaks down food structures, improving the chemical extractability from the matrix. The degree of changes in carotenoids upon processing is dependent upon the type of vegetables, method, temperature and time conditions (Walsh, Bartlett, and Eperjesi 2015).

Therefore, not only nutritional values, but also organoleptic characteristics change very much with cooking. For this reason, many researchers are looking for new cooking techniques to make them safe and palatable and to preserve nutritional characteristics (Paciulli et al. 2018).

Till today, we commonly used cooking methods as boiling, baking, roasting and frying. Yet now, new techniques have emerged such as sous-vide, full steam, microwave and ohmic heating, besides new non-thermal technologies like irradiation, high hydrostatic pressure, pulse electric fields, ultrasounds, ultraviolet light, pulsed light and cold plasma.

Sous-vide cooking is defined as raw materials or raw materials with intermediate foods that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches. It allows to heat to be efficiently transferred from water or steam to the food; it eliminates the risk of recontamination during storage and prevents evaporative losses of flavour volatiles and moisture during cooking (Baldwin 2012).

Full steam is a method of cooking using steam generated by boiling water continuously. It allows to reduce the consumption of water rather than boiling. Furthermore, as Soares, Carrascosa, and Raposo (2017) showed, steaming is the most efficient process to retain health-promoting compounds in cruciferous vegetables when compared to blanching, boiling or microwaving, because it involves fewer losses of water-soluble compounds.

Microwave uses electromagnetic waves and food materials convert microwave energy into heat based on own dielectric properties. This treatment is usually used with others like drying, heating or sterilization to have more advantages and to improve the uniformity during microwaving (Guo et al. 2017).

Ohmic heating applies electrical current into food sample in order to generate heat inside the food. Obviously, it is necessary to have food materials with an appropriate electrical conductivity (range of 0.01-10 S/m) to use this thermal treatment (Jittanit et al. 2017).

Irradiation consists in the application of gamma radiations that damage DNA and inactivate living cells. This non thermal treatment was approved by FDA to inhibit sprouting (using doses between 0.05 and 0.15 KGy). A research conducted on tubers showed that by applying 1 KGy of irradiation decrease the concentration of pesticides, too (Dourado et al. 2019).

High-hydrostatic pressure (HHP) is based on the instantaneous and almost uniform application of high pressures (100-800MPa) to solid or liquid, not to dry or porous foods. HHP has the same efficacy as conventional pasteurization without modifying nutritional and sensory properties of product (Morales-de la Peña, Welti-Chanes, and Martín-Belloso 2019).

Pulse electric fields involves the applications of short pulses (μs to ms) of electric fields in the range of 20-80 kV/cm. It causes microbial death as a result of disintegration of cell membrane for electroporation. It retains or minimally modifies sensorial, nutritional and health-promoting attributes of food products (Dourado et al. 2019).

Ultrasounds utilizes acoustic waves to kill microorganisms. Yet, if it is applied alone, it does not achieve 5 decimal reductions; so, ultrasounds are more effective when combined with temperature (thermo-sonication), pressure (mano-sonication) or both (mano-thermo-sonication).

Ultraviolet light has a wave length between 200 to 280 nm. It is capable to disrupt DNA, but it has poor penetration ability. For this reason, it is widely used to surface and water decontamination, not to dense and opaque liquid.

Pulse light (PL) involves the application of intense and short pulses (100-400 μs) of white light (200-1100 nm). It has the same inactivation mechanism of UV, but it is more penetrating than UV light.

Cold plasma is the newest technique that applies electromagnetic fields to gas (usually O_2 or N_2), generating a mixture of electrons, ions, atoms, UV photons and charged particles that react with food substrate. The accumulation of charged particles induces apoptosis,

electrostatic disruption and electroporation. So, cold plasma has an high efficiency against pathogens, spores and virus (Morales-de la Peña, Welti-Chanes, and Martín-Belloso 2019).

1.9 The case of coloured cauliflower

Cauliflower is a variety of *Brassica Oleracea* var *botrytis*. There are many kinds of cauliflower and each one is characterized by different colour of inflorescence: white, green, purple and orange, as Table 3 shows below. The consumption of this vegetable usually requires a heating treatment, which we will have a positive or negative effect on bioactive compounds, including carotenoids.

Table 3. Varieties of cauliflower

White	Green	Purple	Orange
Snow Cloud	Emeraude	Graffiti	Cheddar
Snowball	Vitaverde	Violetta	Orange Burst
Cloud	Green Macerata	Purple of Sicily	Sunset
Aviso	Monte Verde	Mulberry	

Focused on orange and purple cauliflower, they were born as spontaneous mutation.

The first was discovered in Canada in 1970, it was smaller and less tasty than white one, but its colour was alluring. It took eight years to develop the right germplasm, while other researcher evaluated the nutritional value (Zakour and McCandless 2004). Furthermore, it was discovered that the presence of Or gene perturbs the normal carotenogenesis and the main carotenoids accumulated is β-carotene, that can reach great levels and turn orange (Li et al. 2001).

In the 1980s, purple cauliflower was found in Denmark. Its particular colour is due to a gene mutation that allows anthocyanin accumulation in curds and few other tissues of plant. Its flavour is sweeter and less bitter than normal white cauliflower, so they are more appreciate (Chiu et al. 2010; “Purple Cauliflower” 2011).

In general, cauliflower is native of Mediterranean region, especially of Turkey.

Nowadays, the world’s largest producer of cauliflower is China with about 8.3 million tons (46% of world production), followed by India with 5 million tons (28%) and Italy with 455.000 tons (2.3%). In Europe, the main producers of cauliflower are Italy (455.000 tons), Spain (440.000 tons), France (417.000 tons) and Poland (275.000 tons) (Fao, Istat and Eurostat data).

Brassica vegetables are considered the best food to consume thanks to chemopreventive properties. Many studies associated their consumption to decrease risk of common cancers,

too. These plants are known to have a wide spectrum of bioactive components: glucosinolates, isothiocynates, flavonoids, vitamins and minerals (Koss-Mikołajczyk et al. 2019). Nowadays, scientific community is also giving importance to minority components as: polyphenols and carotenoids that are found in considerable quantities in purple and orange cauliflower, respectively.

Focused on carotenoids, their behaviour is vegetable dependent, so their concentration can increase or decrease. Moreover, cooking plays an important role on their quantity.

2. The aim of thesis

The aim of this research consists in evaluating the effect of thermal treatment on carotenoids in orange and violet cauliflower. The investigated cooking techniques are: boiling, full steaming and sous-vide, as they represent the most common cooking techniques (traditional and innovate) for vegetables in consumers nowadays habits.

3. Materials and methods

3.1 Chemicals and reagents

Standards of β-carotene and lutein (>95% purity) and the solvents used as acetone, acetonitrile, ammonium acetate, dichloromethane and methanol were obtained from Sigma-Aldrich. All the solvents have >95% of purity. Furthermore, H_2O was purified with Millipure System.

3.2 Samples

6 kg of cauliflowers, orange and violet respectively, were collected on 19 November 2018 at the company Agrinovana S.r.l Petritoli, Fermo, Italy). The cauliflower rosettes after cleaning were cut into small pieces of 6-10 g, homogenized and divided into 27 portions of 180-200g.

3.3 Cooking tests

Cauliflower samples were submitted to 3 cooking techniques: boiling, full steaming and sous-vide cooking.

Boiling: cauliflowers were cooked when water (1.5L) was boiling for 10 and 25 minutes.

Full steaming and *sous-vide*: cauliflowers were placed in the special glass container for full steam cooking; while, for sous vide cooking, they have been put in heat-stable vacuumed pouches closed with Combivac sealer drawer EVD14900OX (Electrolux, IT). After, they were placed inside the oven BS8354801M (AEG, Germany) with LXI Data Acquisition Switch Unit (Keysight, US) sensors. Programs were activated at 95°C for 10-25-40 minutes.

Figure 11. At left the oven used for full steam and sous vide cooking; at right LXI Data Acquisition sensors.

In all there were 27 tests: 1 raw, 2 boiling, 3 full steaming and 3 sous-vide, all repeated three times. Once cooked, cauliflowers were cooled down and freeze-dried with Virtis Wizard 2.0 (SP Industries, NY). The lyophilizate is vacuum packed and frozen at -18°C.

3.4 Carotenoids extraction

The extraction was performed according with Biswas, Sahoo, and Chatli (2011). A representative portion of sample (1g) was accurately weighted in a glass test tube. Then 5 ml of chilled acetone was added to it, and the tube was held for 15 min at 4±1°C, vortexed at high speed for 10 min and, finally centrifugated in Neya 16R (Remi Elektrotechnik LTD, India) at 1370 rpm for 10 min (4°C). Supernatant was collected into a separate test tube and, the sample was re-extracted with 5 mL of acetone followed by centrifugation once again as above. Both supernatants were pooled together and filter through Sartorius regenerate cellulose 0.45 µm. The sample was taken to dryness with Rotavapor R-210 (Buchi, Switzerland) and resuspended in 0.5 mL acetone for orange while for violet 0.25 ml.

Figure 12. At left Neya 16R centrifuge; at right rotavapor Buchi.

3.5 Calibration curves

Calibration curve of β-carotene was created using standard diluted in acetone at concentration from 0.054 to 108 µg/ml (R^2=0.9982). On the other hand, the range of lutein's curve was from 0.125 to 100 µg/ml (R^2=0.9918). For the quantification of α-carotene was used the same calibration curve of β-carotene, as they have a similar response.

3.6 UPLC analysis

The analysis was run on Acquity Ultra Pressure Liquid Chromatographic H-class System (Waters Corporation, Milford, US), equipped with a quaternary solvent manager, a sample manager, a column heater and a Photodiode Array (PDA) detector. The whole configuration was driven by Empower software v2.0 from Waters Corporation. A faster version (20 min rather than 46 min) of the method developed by (Duriot et al., 2010) was applied. The stationary phase was the column Acquity UPLC BEH C18 (2.1mm x 100mm, 1.7µm). The mobile phase consisted of phase A, acetonitrile (75)-dicloromethane (10)-methanol (15), and phase B, acetate ammonium in water. The gradient consisted of 75:25 (A:B) from 0 to 10 min, , 98:2 (A:B) from 10 to 11 min, 98:2 (A:B) from 11 to 20 min. Flow rate was 0.4ml/min, column oven set at 35°C and sample loading carried out at 20°C. PDA analysis was performed at 450 nm wavelength upon a spectrum scanning in the 210-500 nm range). Carotenoids were identified by comparison of retention time with pure standards. Their quantification was performed by external calibration (see 3.5).

Table 4. Limit of quantification (LOQ) and Limit of detection (LOD) of lutein, β- and α-carotene in UPLC analysis with column BEH C18.

UPLC analysis	column	carotenoids	LOQ	LOD
			mg/kg dried	
UPLC/PDA	BEH C18	lutein	2.7	0.9
		β-carotene	0.02	0.006
		α-carotene	0.02	0.006

3.7 Statistical analysis

The data were analysed by one-way ANOVA ($p \leq 0.05$) by Tukey test with the software R Project for Statistical Computing.

4. Results and Discussion

4.1. Qualitative analysis of carotenoids in cauliflower

Figure 13 reports a UPLC profile of carotenoids in orange and violet cauliflower. The compounds were recognized by comparing the retention times of pure standards with the sample and by confirmation of PDA spectrum. Lutein, β-carotene and α-carotene were identified and for each compound a calibration curve was implemented to perform carotenoids quantification (see 3.5). However, the phytochemical profile of vegetables is related to environmental and agronomic conditions. Moreover, cauliflower varieties usually display a great variability in carotenoid profile, which can be influenced also by agronomic organic systems (Fibiani 2017).

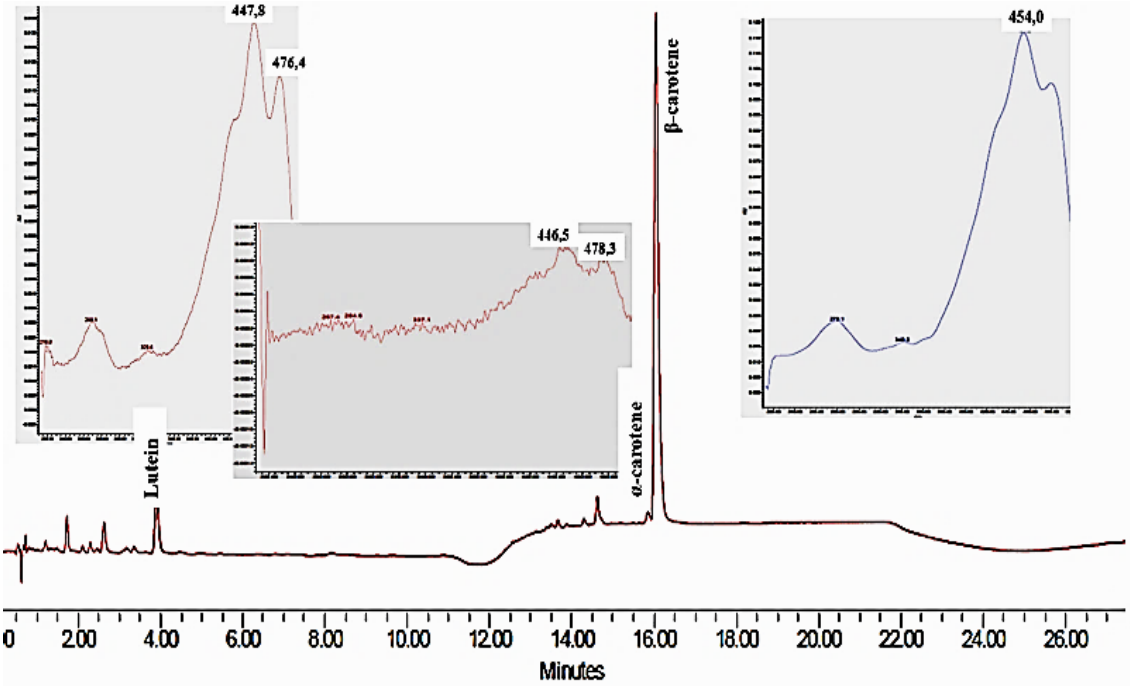


Figure 13. Peaks of lutein, α- and β- carotene in a characteristic chromatogram with the respective absorption spectra.

4.2. Quantitative analysis of carotenoids in cauliflower

In all samples (raw and cooked), the most present carotenoid was lutein, followed by β-carotene and α-carotene. The results differed in part from those of Guzman, Yousef, & Brown (2012) as lutein and α-carotene in raw "Cheddar" samples were not quantified, considering that the extraction method was different. Moreover, neoxanthin and violaxanthin were found in Cheddar and purple cauliflower.

In raw samples, orange and purple cauliflower showed different concentration of carotenoids. Lutein displayed values of 72.9±22.8 and 47.2±21.8 mg/kg of dried sample,

respectively. β-carotene shows values of 20.51±6.71 and 0.94±0.38. α-carotene 0.16±0.12 mg/kg, while for purple cauliflower the value is under the limit of detection. These results were expected as orange cauliflower is a variety where the gene Or is mutated. This is responsible for the over presence of carotenoids that are synthesize and stored in chloroplast (Li et al. 2001). In particular, the level of β-carotene was around 22 times higher, while lutein was 1.5 times higher than purple cauliflower. Furthermore, the ratio of β-carotene/lutein in raw orange cauliflower (0.29±0.01 mg/kg) is different from raw purple one (0.02±0.00 mg/kg).

Table 5. Concentration (mg/Kg) of lutein, β-carotene, α-carotene, total carotenoids and the ratio β-carotene/lutein with standard deviation respectively for each sample. Mean of three replicates ± standard deviation. Different superscript letters in the same column and same cauliflower variety indicate statistically significant difference ($p<0.05$).

sample ID	lutein	β-carotene	α-carotene	total	β-car/lut
Orange Cauliflower					
CAC	72.9±22.8^{a}	20.51±6.71^{a}	0.16±0.12^{a}	93.58±24.98^{a}	0.29±0.01^{a}
CAB10	687.0±101.3^{d}	180.34±18.53^{e}	2.52±0.53^{d}	869.82±108.42^{d}	0.27±0.04^{a}
CAB25	979.0±4.9^{e}	268.51±13.41^{f}	4.03±0.34^{e}	1251.54±18.62^{e}	0.27±0.01^{a}
CAFS10	330.4±30.1^{bc}	99.71±4.84^{bc}	1.33±0.27^{bc}	431.42±32.51^{bc}	0.30±0.03^{a}
CAFS25	412.8±10.7^{bc}	135.60±7.24^{d}	1.36±0.06^{bc}	549.76±12.78^{c}	0.33±0.02^{a}
CAFS40	385.7±51.0^{bc}	142.75±12.02^{d}	1.90±0.36^{cd}	530.30±49.38^{c}	0.37±0.06^{a}
CASV10	262.6±34.1^{b}	67.56±1.18^{b}	0.92±0.01^{ab}	220.71±192.55^{b}	0.26±0.04^{a}
CASV25	395.9±58.5^{bc}	123.10±12.89^{cd}	1.33±0.34^{bc}	520.35±71.70^{c}	0.31±0.01^{a}
CASV40	424.0±12.8^{c}	142.18±2.74^{d}	1.49±0.19^{bc}	567.67±14.20^{c}	0.34±0.01^{a}
Violet Cauliflower					
CVC	47.2±21.8^{a}	0.94±0.38^{a}	<LOD	48.11±22.15^{a}	0.02±0.00^{a}
CVB10	191.2±17.1^{c}	3.72±0.24^{d}	<LOQ	194.93±17.18^{c}	0.02±0.00^{a}
CVB25	251.9±36.1^{d}	4.44±0.24^{d}	<LOQ	256.35±36.32^{d}	0.02±0.00^{a}
CVFS10	86.1±17.4^{ab}	2.03±0.38^{bc}	<LOD	88.16±17.81^{ab}	0.02±0.00^{a}
CVFS25	108.5±3.1^{b}	2.35±0.38^{c}	<LOD	110.88±3.20^{b}	0.02±0.00^{a}
CVFS40	85.7±13.8^{ab}	2.04±0.32^{bc}	<LOD	87.70±14.11^{ab}	0.02±0.00^{a}
CVSV10	55.1±11.3^{a}	1.19±0.28^{ab}	<LOD	56.26±11.61^{a}	0.02±0.00^{a}
CVSV25	79.1±11.2^{ab}	1.69±0.09^{ac}	<LOD	80.74±11.31^{ab}	0.02±0.00^{a}
CVSV40	108.0±8.1^{b}	1.95±0.54^{bc}	<LOD	109.99±8.30^{b}	0.02±0.00^{a}

Our outcomes are in accord with previous studies, reporting in the same orange cauliflower variety with β-carotene amounts ranging from 5 to 8 mg/kg fresh weight (approximately 33−53 mg/kg dried weight) and negligible amounts of neoxanthin, violaxanthin, and lutein (Li et al. 2001).

4.2.1 Temperature effect

The temperature of thermal process (95°C for full steam and sous vide, 100°C for boiling) had a positive influence on the extractability of carotenoids, as all cooked samples showed higher value when compared to raw samples. In both cauliflower (orange and violet), the highest total carotenoid content was revealed sample boiled for 25 minutes (CAB25 and CVB25).

It is interesting to notice that the ratio β-carotene/lutein in both cauliflowers did not vary in any cooking test, suggesting that lutein (polar, xanthophyll) and β-carotene (apolar, carotene) responds in the same way to heat treatments around 95-100°C.

Reporting the results for each carotenoid into diagrams (Figure 16), all cooking methods increase the concentration of carotenoids. According to Walsh et al. (2015), heat treatments increase the bioavailability of carotenoids as it inactivates enzymes, breaks down food structure and the protein-carotenoids complexes. On the other hand, in white cauliflower some authors found a different behavior (higher or lower extraction) related to the type of carotenoid (lutein, β-carotene, α-carotene, zeaxanthin and β-cryptoxanthin) at different thermal treatments (boiling, steaming, sous vide and microwaving), Table 6 (Dos Reis et al. 2014).

4.2.2. Boiling

Different cooking techniques has different effect on carotenoids content.

Figure 15 showed boiling as the best cooking method when compared to full steam and sous-vide cooking. CAB25 reached a maximum value of lutein, b-carotene and a-carotene 979.0±4.9, 268.51±13.41 and 4.03±0.34 mg/kg, respectively. CVB25 reached 251.9±63.1 (lutein), 4.44±0.24 mg/kg (β-carotene) and α-carotene was at least detected. For each carotenoid, the maximum was detected after 25 minutes of treatment even if the difference between raw samples and boiled for 10 minutes is greater than between boiled for 10 and 25 minutes.

The big quantity of carotenoids contained in orange cauliflower makes process of boiling better than the others. On the other hand, purple cauliflower is richer in polyphenols than carotenoids, so even if boiling increases carotenoid content, it could reduce polyphenols content (because they are water-soluble).

4.2.3. Full steamed samples

The temperature of thermal process (95°C) had a positive influence on the extractability of carotenoids. The maximum values of total carotenoids in full steam cooking were 549.76±12.78 mg/kg (orange) and 110.88±3.20 mg/kg dried (purple), reached after 25 minutes.

This is in contrast with Pellegrini et al., (2010), which stated (about *Brassica* vegetables) that steam process did not affect carotenoid content.

Furthermore, the quantity of lutein, β-carotene and α-carotene (in orange and purple cauliflower) at 25 minutes of heating was the same obtained after 40 minutes of sous-vide cooking.

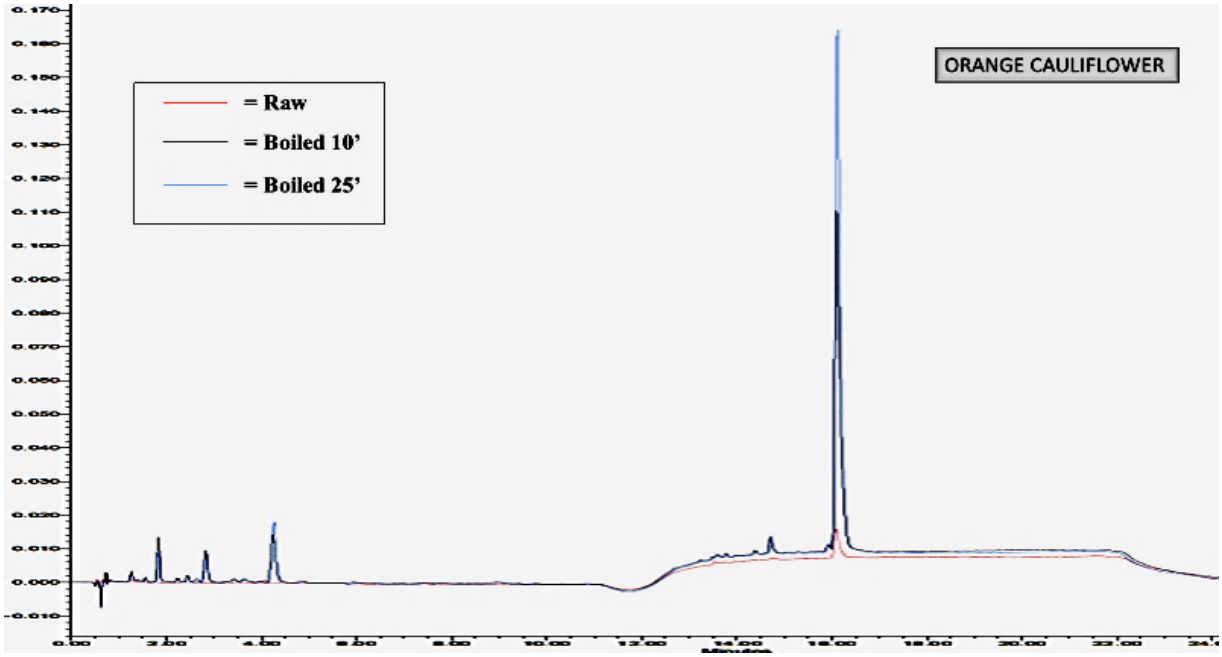
4.2.4. Sous-vide samples

Making in comparison full steam and sous-vide samples, they had a similar trend, as they displayed similar values. Sous-vide reached the maximum concentration of 567.67±14.20 (orange) and 109.99±8.30 mg/kg dried (purple), after 40 minutes.

Moreover, for each carotenoid (lutein, β-carotene, α-carotene and also total carotenoid) the only significant difference was between sous-vide treatment at 10 and 40 minutes. Disagree with Dos Reis et al., (2014) (Table 6) as lutein and β-carotene found in sous-vide samples (of white cauliflower) after 20 minutes of cooking were less than raw samples. Our outcomes revealed an increase of about 5.4 and 6 times respectively, in orange cauliflower after 25 minutes of cooking. On the other hand, in purple cauliflower CVSV25 were not significantly different from raw samples.

Table 6. Analysis of carotenoids in different processes (µg/ 100g dry weight; mean of 3 replicates and standard deviation) (Dos Reis et al. 2014)

	Organic cauliflower				
	Fresh	Boiling	Steam	Microwave	*Sous vide*
Lutein	34.18 ± 1.42^{a}	59.79 ± 0.62^{b}	121.10 ± 1.13^{c}	54.57 ± 1.01^{d}	22.86 ± 0.97^{e}
Zeaxanthin	3.83 ± 0.04^{d}	6.99 ± 0.15^{b}	16.65 ± 0.65^{a}	$6.32 \pm 0.33^{b,c}$	$5.15 \pm 0.12^{c,d}$
Cryptoxanthin	$15.90 \pm 0.63^{b,c}$	19.22 ± 0.85^{b}	31.71 ± 1.00^{a}	$14.56 \pm 0.18^{c,d}$	12.61 ± 0.68^{d}
α-carotene	3.75 ± 0.20^{b}	2.89 ± 0.17^{c}	9.70 ± 0.25^{a}	3.66 ± 0.15^{b}	2.17 ± 0.02^{d}
β-carotene	556 ± 27.94^{a}	135 ± 6.41^{b}	163 ± 13.02^{b}	76.57 ± 3.23^{c}	45.03 ± 3.12^{c}
Total carotenoids	614 ± 30.23^{a}	224 ± 8.20^{c}	342 ± 14.05^{b}	156 ± 4.53^{d}	87.84 ± 2.69^{e}
Vitamin A	42.81 ± 2.15^{a}	10.41 ± 0.49^{b}	12.53 ± 1.00^{b}	5.89 ± 0.25^{c}	3.46 ± 0.24^{c}



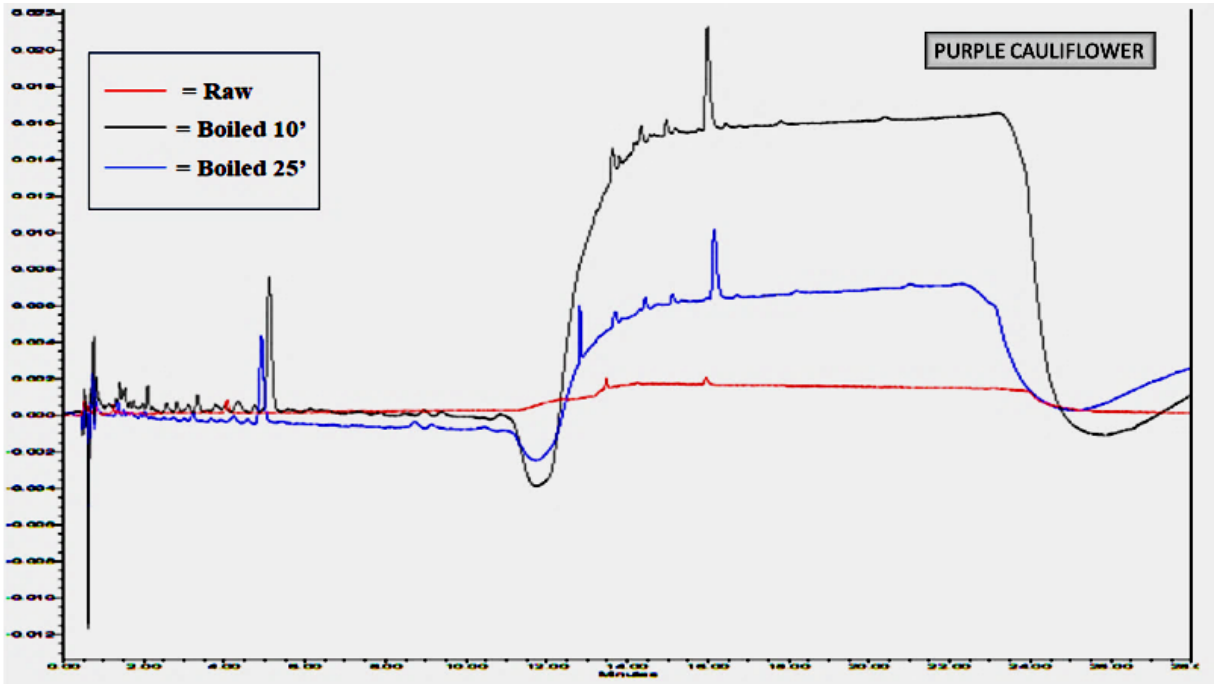


Figure 14. Compared chromatograms at different cooking times: 0, 10 and 25 minutes of orange cauliflower (up) and purple cauliflower (down)

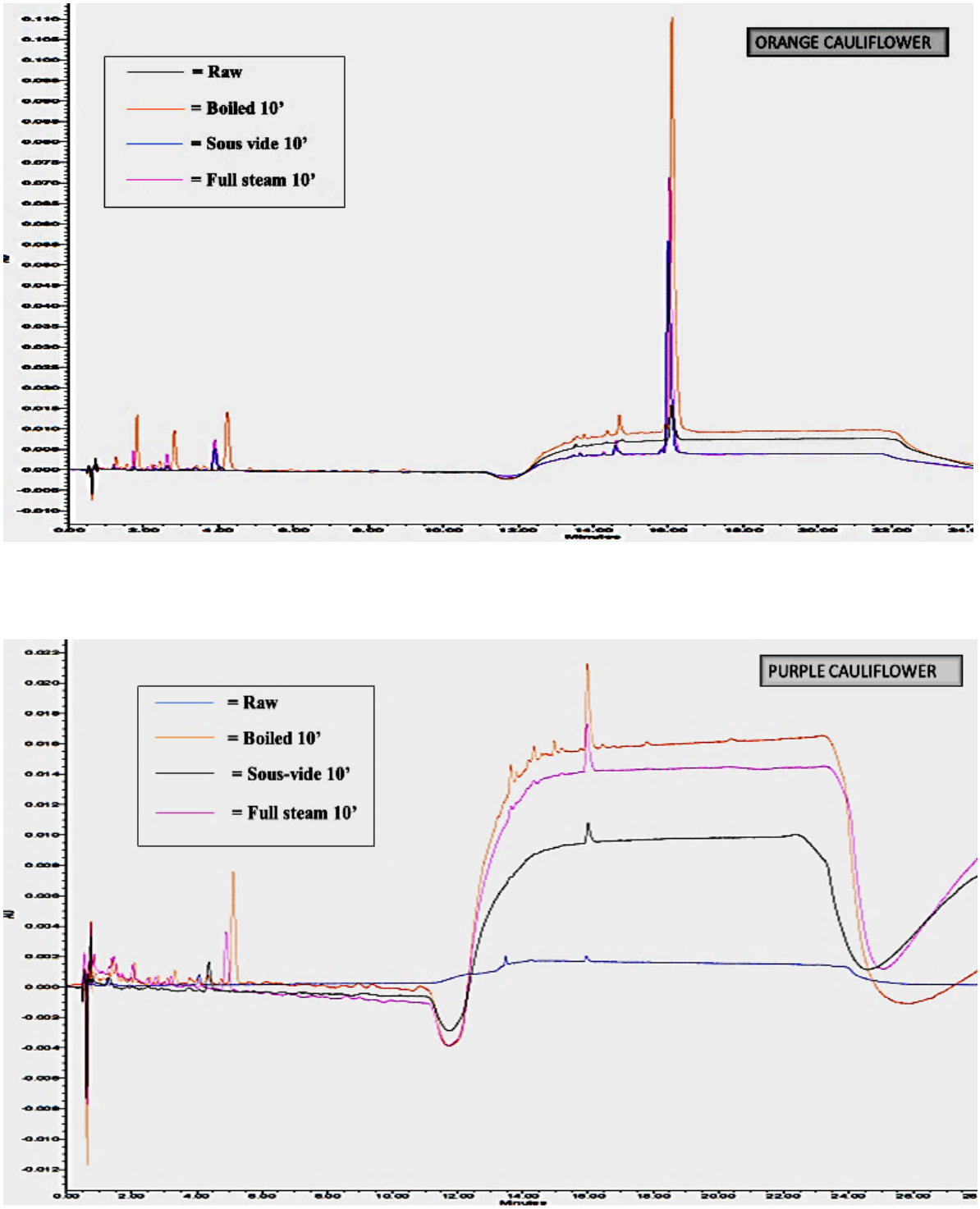


Figure 15. Compared chromatograms at the same cooking time of different cooking techniques (boiled, full steam, sous-vide) and the raw one of orange cauliflower (up) and purple cauliflower (down).

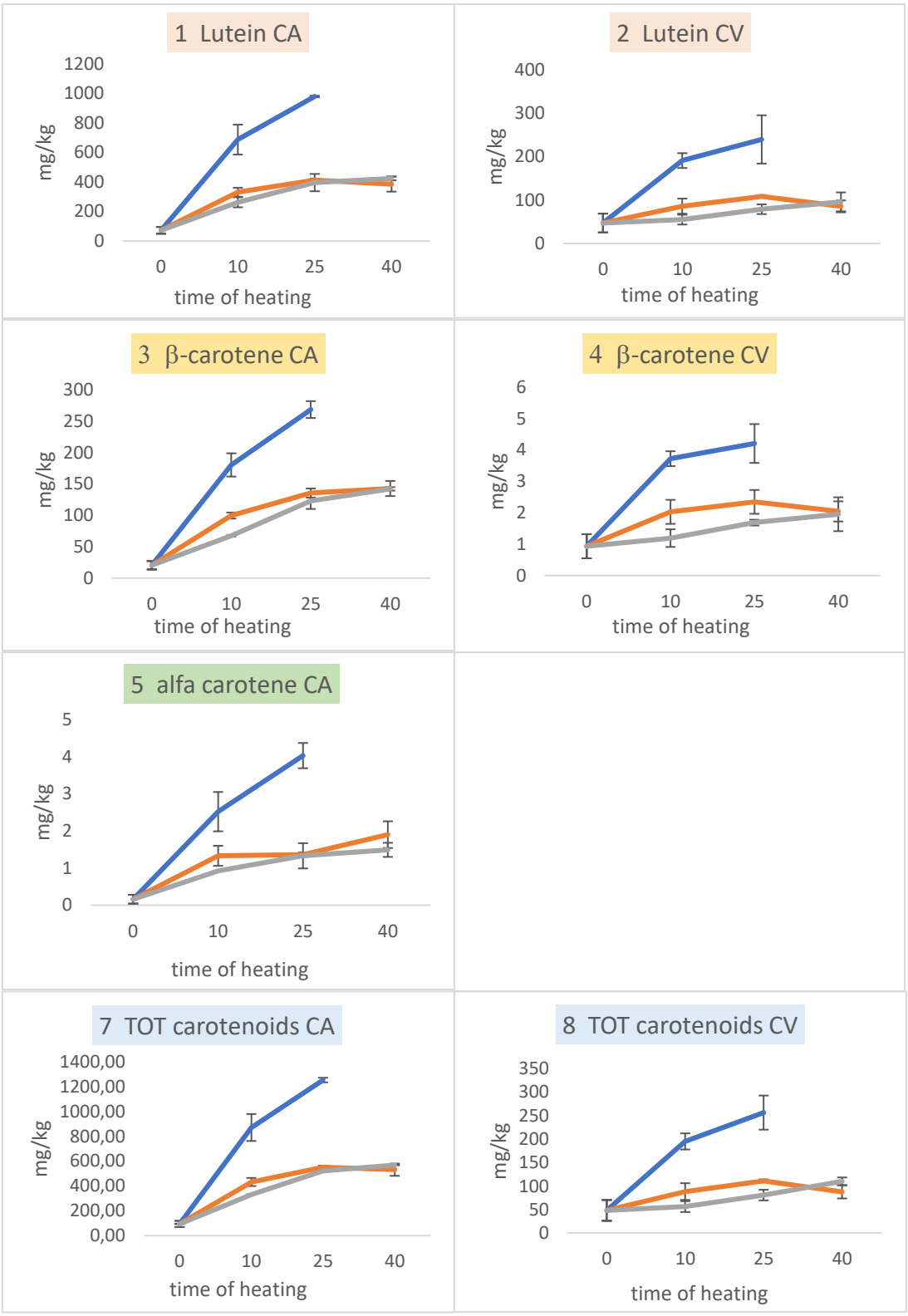


Figure 16. Evolution of lutein, β- and α- carotene and, total carotenoids (mg/kg dried) in orange (CA) and violet (CV) cauliflower cooked with 3 different techniques: boiling (blu), steamed (orange), sous-vide (grey) for different times of heating (0, 10, 25, 40). Values are means of three replicates±standard deviation.

5. Conclusion

Carotenoids are very widespread, especially in fruits and vegetables. Cauliflowers (in particular “Cheddar” variety) have a great amount of them and the most common carotenoids are lutein, β-carotene and α-carotene. All thermal treatment allows to increase the extractability and bioavailability of carotenoids. Among boiling, full steam and sous-vide cooking, the first appeared to be the best, because it demonstrated higher concentration of carotenoids (after 25 min of cooking): 1251.54±18.62 (orange) and 256.35±36.32 (violet). Steaming and sous-vide cooking displayed similar values. In sous-vide samples, carotenoid content increased with the time of heating. On the contrary, in steaming samples this is not always true: some carotenoids decreased with longer time of heating (at 40 min).

Bibliography

Baldwin, Douglas E. 2012. "Sous Vide Cooking: A Review." *International Journal of Gastronomy and Food Science* 1 (1): 15–30. https://doi.org/10.1016/j.ijgfs.2011.11.002.

Barba, Francisco J., Lilian R.B. Mariutti, Neura Bragagnolo, Adriana Z. Mercadante, Gustavo V. Barbosa-Cánovas, and Vibeke Orlien. 2017. "Bioaccessibility of Bioactive Compounds from Fruits and Vegetables after Thermal and Nonthermal Processing." *Trends in Food Science and Technology* 67: 195–206. https://doi.org/10.1016/j.tifs.2017.07.006.

Biswas, A. K., J. Sahoo, and M. K. Chatli. 2011. "A Simple UV-Vis Spectrophotometric Method for Determination of β-Carotene Content in Raw Carrot, Sweet Potato and Supplemented Chicken Meat Nuggets." *LWT - Food Science and Technology* 44 (8): 1809–13. https://doi.org/10.1016/j.lwt.2011.03.017.

Britton, George, S. Liaaen-Jensen, and Hanspeter Pfander. 2004. *Carotenoids Handbook*.

Chiu, L.-W., X. Zhou, S. Burke, X. Wu, R. L. Prior, and L. Li. 2010. "The Purple Cauliflower Arises from Activation of a MYB Transcription Factor." *Plant Physiology* 154 (3): 1470–80. https://doi.org/10.1104/pp.110.164160.

Cilla, Antonio, Lourdes Bosch, Reyes Barberá, and Amparo Alegría. 2018. "Effect of Processing on the Bioaccessibility of Bioactive Compounds – A Review Focusing on Carotenoids, Minerals, Ascorbic Acid, Tocopherols and Polyphenols." *Journal of Food Composition and Analysis* 68: 3–15. https://doi.org/10.1016/j.jfca.2017.01.009.

Dourado, Cátia, Carlos Pinto, Francisco J. Barba, Jose M. Lorenzo, Ivonne Delgadillo, and Jorge A. Saraiva. 2019. "Innovative Non-Thermal Technologies Affecting Potato Tuber and Fried Potato Quality." *Trends in Food Science and Technology* 88 (February): 274–89. https://doi.org/10.1016/j.tifs.2019.03.015.

Duriot, B. Chauveau. 2010. "Simultaneous Quantification of Carotenoids, Retinol, and Tocopherols in Forages, Bovine Plasma, and Milk: Validation of a Novel UPLC Method."

Eisenhauer, Bronwyn, Sharon Natoli, Gerald Liew, and Victoria M. Flood. 2017. "Lutein and Zeaxanthin — Food Sources, Bioavailability and Dietary Variety in Age-related Macular Degeneration Protection." *Nutrients* 9 (2). https://doi.org/10.3390/nu9020120.

Fennema, Owen R. 1996. *Food Chemistry*. Edited by CRC Press. 3rd ed. Marcel Dekker.

Fibiani, Marta. 2017. "Qualità Nutrizionale Del Cavolfiore Biologico: Risultati Di Dieci Anni Di Prove Sperimentali. (Nutritional Quality of Organic Cauliflower: Ten-Year Results of Field Trials)," no. June.

Food Standards Australia New Zealand. 2017. “Food Derived from Provitamin A Rice Line GR2E.”

Guo, Qiushan, Da Wen Sun, Jun Hu Cheng, and Zhong Han. 2017. “Microwave Processing Techniques and Their Recent Applications in the Food Industry.” *Trends in Food Science and Technology* 67: 236–47. https://doi.org/10.1016/j.tifs.2017.07.007.

Guzman, Ivette, Gad G. Yousef, and Allan F. Brown. 2012. “Simultaneous Extraction and Quantitation of Carotenoids, Chlorophylls, and Tocopherols in Brassica Vegetables.” *Journal of Agricultural and Food Chemistry* 60 (29): 7238–44. https://doi.org/10.1021/jf302475d.

Hoang, Dong Van, Ngoc Minh Pham, Andy H. Lee, Duong Nhu Tran, and Colin W. Binns. 2018. “Dietary Carotenoid Intakes and Prostate Cancer Risk: A Case-Control Study from Vietnam.” *Nutrients* 10 (1): 1–11. https://doi.org/10.3390/nu10010070.

Jittanit, Weerachet, Krittiya Khuenpet, Pattamaporn Kaewsri, Nunthawan Dumrongpongpaiboon, Pawisa Hayamin, and Kornkanok Jantarangsri. 2017. “Ohmic Heating for Cooking Rice: Electrical Conductivity Measurements, Textural Quality Determination and Energy Analysis.” *Innovative Food Science and Emerging Technologies* 42 (January): 16–24. https://doi.org/10.1016/j.ifset.2017.05.008.

Koss-Mikołajczyk, Izabela, Barbara Kusznierewicz, Wiesław Wiczkowski, Natalia Płatosz, and Agnieszka Bartoszek. 2019. “Phytochemical Composition and Biological Activities of Differently Pigmented Cabbage (Brassica Oleracea Var. Capitata) and Cauliflower (Brassica Oleracea Var. Botrytis) Varieties.” *Journal of the Science of Food and Agriculture*, no. December 2018. https://doi.org/10.1002/jsfa.9811.

Lachman, Jaromír, K. Hamouz, M. Orsák, and Z. Kotíková. 2016. “Carotenoids in Potatoes - A Short Overview.” *Plant, Soil and Environment* 62 (10): 474–81. https://doi.org/10.17221/459/2016-PSE.

Li, Li, Dominick J. Paolillo, Mandayam V. Parthasarathy, Elena M. DiMuzio, and David F. Garvin. 2001. “A Novel Gene Mutation That Confers Abnormal Patterns of β-Carotene Accumulation in Cauliflower (Brassica Oleracea Var. Botrytis).” *Plant Journal* 26 (1): 59–67. https://doi.org/10.1046/j.1365-313X.2001.01008.x.

Liu, Rui Hai. 2013. “Health-Promoting Components of Fruits And.” *Advances in Nutrition* 4: 384S–392S. https://doi.org/10.3945/an.112.003517.convenient.

Melèndez-Martìnez, Antonio J. 2016. “Newsletter Carotenoids : Ancient and Widespread Versatile Compounds for Agro-Food and Health,” 1–8.

Mignogna, Rossella. 2010. “Valutazione Di Composti Bioattivi Ad Attività Antiossidante in

Alimenti Di Origine Vegetale." University of Molise.

Morales-de la Peña, M., J. Welti-Chanes, and O. Martín-Belloso. 2019. "Novel Technologies to Improve Food Safety and Quality." *Current Opinion in Food Science* 30: 1–7. https://doi.org/10.1016/j.cofs.2018.10.009.

Paciulli, Maria, Chiara Dall'Asta, Massimiliano Rinaldi, Nicoletta Pellegrini, Alessandro Pugliese, and Emma Chiavaro. 2018. "Application and Optimisation of Air–Steam Cooking on Selected Vegetables: Impact on Physical and Antioxidant Properties." *Journal of the Science of Food and Agriculture* 98 (6): 2267–76. https://doi.org/10.1002/jsfa.8715.

Paolo, Cabras, and Martelli Aldo. 2004. *Chimica Degli Alimenti*. Piccin.

Pellegrini, Nicoletta, Emma Chiavaro, Claudio Gardana, Teresa Mazzeo, Daniele Contino, Monica Gallo, Patrizia Riso, Vincenzo Fogliano, and Marisa Porrini. 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 (7): 4310–21. https://doi.org/10.1021/jf904306r.

"Purple Cauliflower." 2011. Mansfield city.

Reis, Luzia Caroline Ramos Dos, Viviani Ruffo De Oliveira, Martine Elisabeth Kienzle Hagen, André Jablonski, Simone Hickmann Flôres, and Alessandro De Oliveira Rios. 2014. "Effect of Cooking on the Concentration of Bioactive Compounds in Broccoli (Brassica Oleracea Var. Avenger) and Cauliflower (Brassica Oleracea Var. Alphina F1) Grown in an Organic System." *Food Chemistry* 172: 770–77. https://doi.org/10.1016/j.foodchem.2014.09.124.

Saini, Ramesh Kumar, Shivraj Hariram Nile, and Se Won Park. 2015. "Carotenoids from Fruits and Vegetables: Chemistry, Analysis, Occurrence, Bioavailability and Biological Activities." *Food Research International* 76: 735–50. https://doi.org/10.1016/j.foodres.2015.07.047.

Soares, Ana, Conrado Carrascosa, and António Raposo. 2017. "Influence of Different Cooking Methods on the Concentration of Glucosinolates and Vitamin C in Broccoli." *Food and Bioprocess Technology* 10 (8): 1387–1411. https://doi.org/10.1007/s11947-017-1930-3.

Sourkes, Theodore L. 2009. *The Discovery and Early History of Carotene*. *Bull. Hist. Chem*. Vol. 34. http://www.scs.illinois.edu/~mainzv/HIST/bulletin_open_access/v34-1/v34-1 p32-38.pdf.

Szalay, Jessie. 2015. "Live Science." Live Science. 2015. http://www.livescience.com/1292-history-climate-change-science.html.

Tanumihardjo, Sherry A. 2013. “Carotenoids and Human Health.” *Carotenoids and Human Health* 55: 1–331. https://doi.org/10.1007/978-1-62703-203-2.

Walsh, Rachel P., Hannah Bartlett, and Frank Eperjesi. 2015. “Variation in Carotenoid Content of Kale and Other Vegetables: A Review of Pre- and Post-Harvest Effects.” *Journal of Agricultural and Food Chemistry* 63 (44): 9677–82. https://doi.org/10.1021/acs.jafc.5b03691.

Weaver, Ryan J., Eduardo S.A. Santos, Anna M. Tucker, Alan E. Wilson, and Geoffrey E. Hill. 2018. “Carotenoid Metabolism Strengthens the Link between Feather Coloration and Individual Quality.” *Nature Communications* 9 (1). https://doi.org/10.1038/s41467-017-02649-z.

Zakour, John, and Linda McCandless. 2004. “Orange Cauliflower Developed at Cornell ’ s Experiment Station Is High in Vitamin A,” 1–3.

Zakynthinos, G, and T Varzakas. 2016. “Carotenoids: From Plants to Food Industry.” *Current Research in Nutrition and Food Science Journal* 4 (1): 38–51. https://doi.org/10.12944/crnfsj.4.special-issue1.04.

Thanks

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