DIPARTIMENTO DI SCIENZE AGRARIE ALIMENTARI E AMBIENTALI

CORSO DI LAUREA IN: SCIENZE E TECNOLOGIE ALIMENTARI

EFFECT OF THERMAL TREATMENT ON TOCOPHEROLS IN CAULIFLOWER

EFFETTO DEL TRATTAMENTO TERMICO SUI TOCOFEROLI NEL CAVOLFIORE

TIPO TESI: sperimentale

Studente:
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Riassunto

Il cavolfiore contiene diversi composti bioattivi. Il cavolfiore arancione e viola sono ricchi anche in carotenoidi e polifenoli, rispettivamente. Tra i componenti minoritari e meno studiati si trovano i tocoferoli, che nel cavolfiore sono presenti nella forma γ e α.

Poiché il consumo del cavolfiore prevede un processo di cottura, è importante comprendere il comportamento dei composti bioattivi, quali tocoferoli, in relazione al trattamento termico al fine di preservare e/o aumentare il valore nutrizionale.

Lo scopo del lavoro è stato quello di dimostrare gli effetti della cottura di vari trattamenti termici sui tocoferoli, in una matrice vegetale come il cavolfiore, nelle varietà arancione e viola.

Sono state sperimentate tre tipologie: bollito, cottura a vapore e sottovuoto. Ognuna di queste è stata effettuata a temperature di 10, 25 e 40 minuti, ad eccezione del bollito che è stato sottoposto solamente ai tempi di 10 e 25 minuti.

I tocoferoli sono stati estratti tramite una saponificazione a caldo 85°C per 30 minuti con successiva analisi in UPLC, usando come detector il fluorimetro.

Dai risultati delle analisi è stato possibile osservare che il cavolfiore arancione e il cavolfiore viola hanno profili tocoferolici diversi; il cavolfiore arancione, infatti, presenta, rispetto al viola, anche la forma δ, oltre le forme γ e α.

I risultati hanno mostrato che il bollito risulta una tecnica migliore rispetto alla cottura al vapore e al sottovuoto alle stesse temperature di 10 e 25 minuti (131.8±19.9 mg/kg e 183.4±24.1 mg/kg per la varietà arancione; 87.1±9.5 mg/kg e 134.1±10.2 mg/kg per la varietà viola).

Inoltre, la cottura al vapore e il sottovuoto presentano lo stesso effetto su questi composti bioattivi. Allo stesso tempo, grazie alla cottura a vapore non si ha la degradazione dei componenti, anzi si ha anche una maggiore estraibilità sopratutto nella varietà arancione. Risultato simile è emerso anche dalla cottura del "sous-vide" ovvero dalla cottura del sottovuoto, che secondo alcuni è ritenuta la cottura migliore rispetto le altre.

Dunque, dai risultati delle analisi è emerso che la degradazione dei vari componenti bioattivi, quali ad esempio le vitamine o altri composti, durante diversi trattamenti termici effettuati in tempi differenti, dipende dal tipo di matrice che si tratta.

Questo studio fornisce informazioni sulle tecniche tradizionali e innovative di cottura e mostra quali sono gli effetti sui tocoferoli, composti che sono meno studiati in una matrice come il cavolfiore.

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Acronyms and abbreviations

BAv = bioavailability

BAcs = bioaccssibility

BAct = bioactivity

α-T = α-tocopherol

γ-T = γ-tocopherol

δ-T = δ-tocopherol

α-TTP = α-tocopherol-transfer

ABCA1 = ATP-binding-cassette A1

CEHCs = 3'-carboxychromanol

RDA = Recommended Dietary

HPLC = High Performance Liquid Chromatography

CVC = Cavolfiore viola crudo; CVB10 = Cavolfiore viola bollito a 10 minuti; CVB25 = Cavolfiore viola bollito a 25 minuti; CVFS10 = Cavolfiore viola full steam a 10 minuti; CVFS25 = Cavolfiore viola full steam a 25 minuti; CVFS40 = Cavolfiore viola full steam a 40 minuti; CVSV10 = Cavolfiore viola sous vide a 10 minuti; CVSV25 = Cavolfiore viola sous vide a 25 minuti; CVSV40 = Cavolfiore viola sous vide a 40 minuti.

CAC = Cavolfiore arancione crudo; CAB10 = Cavolfiore arancione bollito a 10 minuti; CAB25 = Cavolfiore arancione bollito a 25 minuti; CAFS10 = Cavolfiore arancione full steam a 10 minuti; CAFS25 = Cavolfiore arancione full steam a 25 minuti; CAFS40 = Cavolfiore arancione full steam a 40 minuti; CASV10 = Cavolfiore arancione sous vide a 10 minuti; CASV25 = Cavolfiore arancione sous vide a 25 minuti; CASV40 = Cavolfiore arancione sous vide a 40 minuti.

KOH = Potassium hydroxide

UPLC = Ultra Pressure Liquid Cromatographic

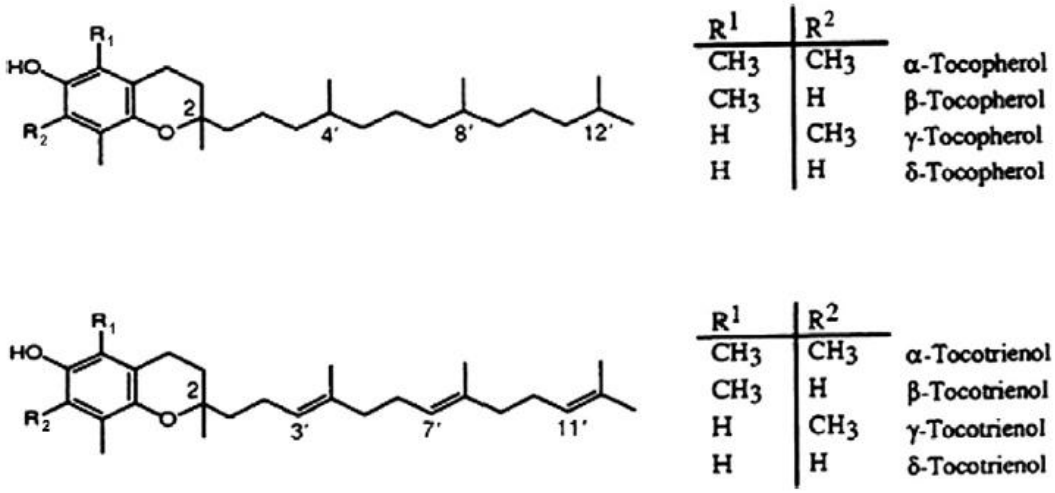
SD = Standard Deviation

1. Introduction

1.1 General Information

Vitamin E is a fat-soluble vitamin known for its antioxidant capacity and health human benefits. It was discovered in 1922 by the scientists Evans and Bishop-being isolated from green vegetables (Peh et al. 2016).

There are eight isoforms of Vitamin E, classified into tocopherols and tocotrienols, with conformations α-, β-, γ-, δ- respectively (Jiang 2014). Tocopherols and tocotrienols share common structure features of a chromanol ring and a side-chain at the C-2 position (Figure 1). The former contains a saturated phytyl tail, whereas the latter bears an unsaturated isoprenoid side-chain (Nesaretnam 2008).The unsaturated isoprenoid side chain of tocopherols potentially enhances its penetration into fatty tissues, such as brain and liver, and better distribution on the cell membranes. Vitamin E consists of the dextrorotatory enantiomers only with a single stereoisomer. Tocopherols contain three chiral stereocenters at C-2, C-4' and C-8', while tocotrienols contain only one chiral stereocenter at C-2 as the other two chiral stereocenters are not possible with C=C unsaturation in the isoprenoid tail (Peh et al. 2016).



R^1	R^2	
CH_3	CH_3	α-Tocopherol
CH_3	H	β-Tocopherol
H	CH_3	γ-Tocopherol
H	H	δ-Tocopherol

R^1	R^2	
CH_3	CH_3	α-Tocotrienol
CH_3	H	β-Tocotrienol
H	CH_3	γ-Tocotrienol
H	H	δ-Tocotrienol

Figure 1. Chemical nature of different members of Vitamin E family. Figure taken from Nesaretnem et al., 2008.

1.2 Health benefits of Vitamin E

Tocopherols are considered as the most effective lipophilic natural antioxidants, which could prevent lipid peroxidation by acting as peroxyl radical scavengers that terminate chain reactions in membranes and lipoprotein particles (Zhang et al., 2014). The predominant reaction responsible for tocopherol antioxidant activity is hydrogen atom donation, where a tocopheroxyl radical is formed (Podsedek 2007). α-tocopherol is considered the main contributor to vitamin E activity, but γ-tocopherol is a more efficient antioxidant (Amarowicz & Pegg, 2008). Recently, tocotrienols have gained attention in cell protection (Nesaretnam, 2008).

The antioxidant activity of vitamin E is also related to its synergistic effect with another antioxidant. Tocopherols and tocotrienols can indirectly act as lipid peroxidation inhibitors, for example, protecting other antioxidants from oxidation. It is suggested that α-tocopherol interact with β-carotene, preserving it from reactive oxygen species (Munnè-Bosh, 2002).

A deficiency of Vitamin E may cause neuromuscular problems, being necessary for the integrity of Purkinje neurons and being associated with greater fat-free mass (Ulatowski et al. 2014). The cerebellar origin of vitamin E-deficiency-induced ataxia, the critical role of Purkinje neurons in projecting cerebellar cortex output and the known sensitivity of cerebellar Purkinje neurons to oxidative stress raise the possibility that integrity of Purkinje neurons is compromised. Vitamin E compounds have also potential clinical applications in multiple diseases as shown in Figure 2 (Peh et al. 2016). For example, they protect cells against oxidative damage, and may therefore prevent chronic diseases, such as cancer, cardiovascular diseases and diabetes (Podsedek 2007).

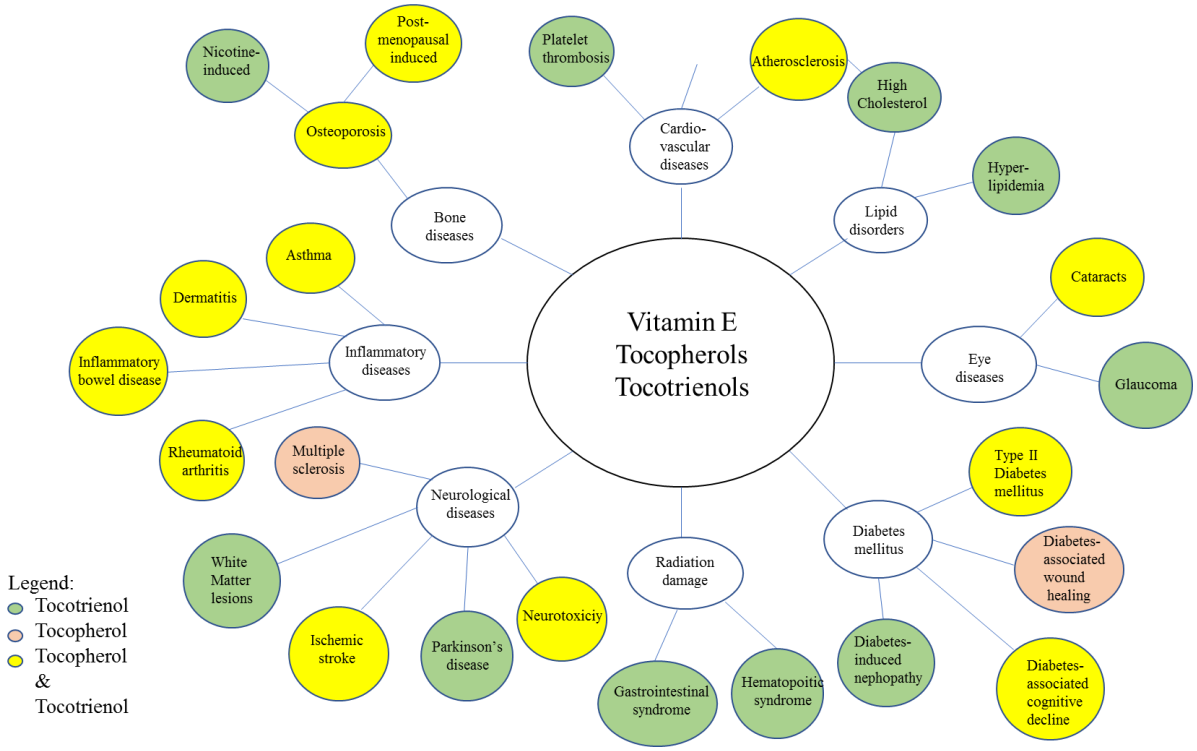


Figure 2 Vitamin E potential clinical applications in multiple diseases. Figure adapted from Peh et al., 2016

1.3 Bioavailability

The potential of bioactive components within foods to exert their effects in the body depends on their matrix release, changes during digestion, uptake, metabolism and biodistribution. The term bioavailability (BAv) has several working conditions and there is no universally accepted definition. From a nutritional point of view, it is defined as the fraction of ingested component available for utilization in normal physiological function (Cilla et al. 2018).

BAv included two additional terms: bioaccessibility (BAcs) and bioactivity (BAct).

Bioaccessibility has two different definition. The first one is the fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption. The second one is more stringent and much less widely used, described as the fraction of a compound that is released from its food matrix in the gastrointestinal tract becoming available for intestinal absorption, including absorption/assimilation into the cells of the intestinal epithelium and, lastly, pre-systemic intestinal and hepatic metabolism (Cilla et al. 2018).

Bioactivity (BAct) includes events linked to how the bioactive compound has reached systemic circulation and it's transported and reaches the target tissue, interaction with biomolecules metabolism in these tissues, and all the cascade of physiological effects it generates (Cilla et al. 2018).

In the intestine, dietary tocopherols and tocotrienols are secreted in chylomicron particles together with triacylglycerols, phospholipids and cholesterol. The chylomicron-bound vitamin E forms are transported via the lymphatic system to the peripheral tissues, including muscle, bone marrow, adipose tissue, skin, and possibly brain. In these tissues, vitamin E forms are picked up by a lipoprotein receptor-mediated process. Chylomicron-associated tissue uptake of vitamin E may contribute to the accumulation of non-αT forms of vitamin E such as γ-T in human skin, adipose tissue and muscle where high concentration of γ-T was observed, in contrast to its low levels in the plasma. The resulting chylomicron remnants are taken up by the liver (Jiang 2014).

In the liver, α-T is preferentially bound to α-tocopherol-transfer protein (α-TTP). α-TTP, together with ATP-binding cassette transporter A1 (ABCA1), incorporates αT into lipoproteins, which transport vitamin E to other tissue via the circulation (Jiang 2014). α-TTP has a high affinity to αT (100%) and lower for other vitamin E isoforms: approximately 50% for β-tocopherol, 10-30% for γ-tocopherol and 1% for δ-tocopherol (Peh et al. 2016); in fact,

large portions of non-αT forms of vitamin E are catabolized in the liver via cytochrome P450 (CYP4F2)- initiated ω-hydroxylation and oxidation followed by β-oxidation of the phytyl chain to generate 13'-hydroxychromanol (13'- OH), various carboxychromanol, and terminal metabolite 3'-carboxychromanol or (2'-carbox-yethyl)-6-hydroxychromans (CEHCs) (Jiang 2014) (Figure 3).

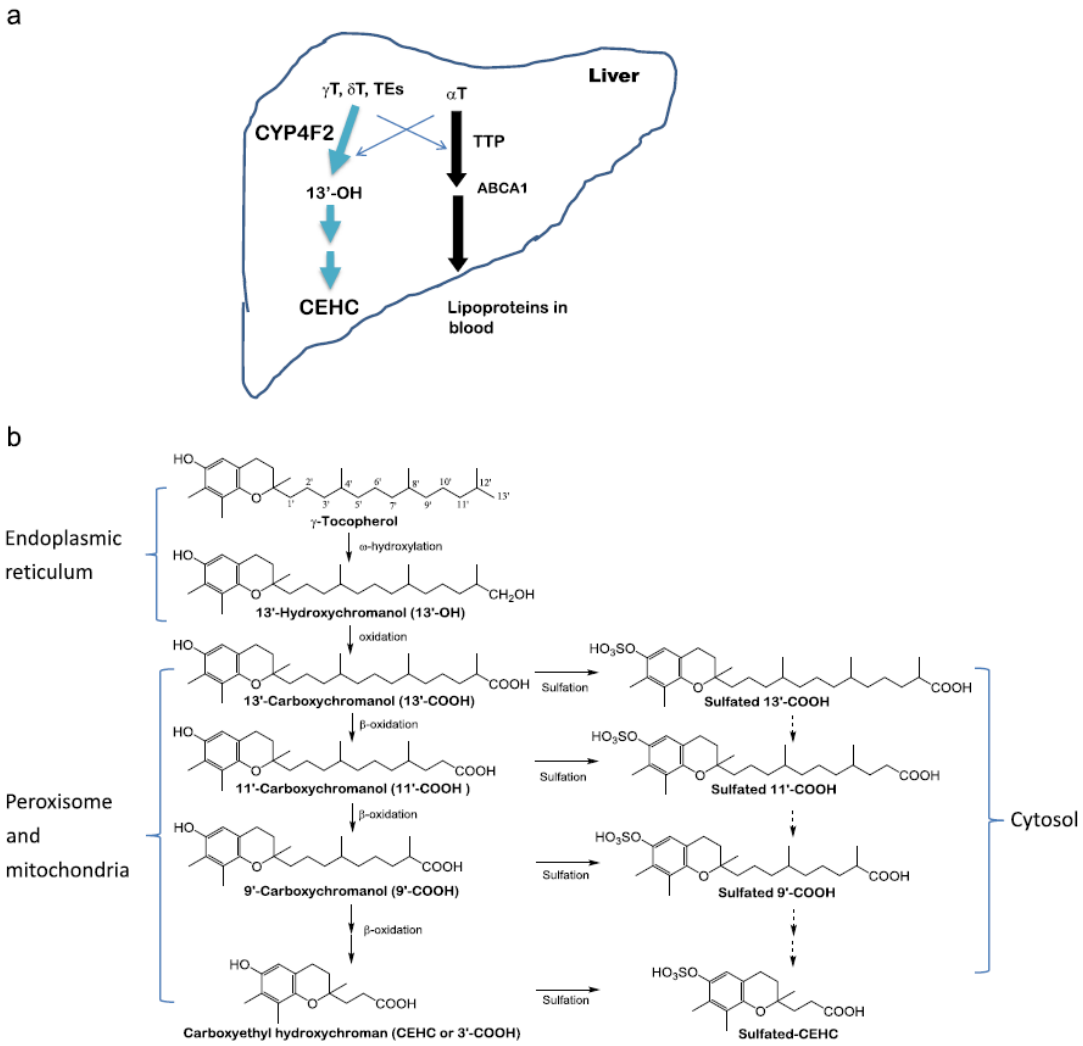


Figure 3. a. Transport and metabolism of vitamin E forms in the liver. b. Molecular mechanism of Vitamin E metabolism. Figure taken from Jiang 2014.

Food processing is one of the main determination on BAv, because it can have a positive or negative impact by increasing or decreasing BAcs of nutrients and bioactive compounds, respectively (Cilla et al. 2018).

To determinate bioaccessibility there are two methods: *in vivo* and *in vitro*.

In vitro models are cost-effective, reproducible and rapid methods that can be used to determinate the effect of food matrix, processing methods, and dietary components on BAcs of bioactive compounds in foods and are used as surrogates of *in vivo* studies for predictive purposes.

For each nutrient or bioactive compound, specific *in vitro* digestion models must be tailored, changed, tested and validated against *in vivo* studies. It can not be taken for granted that *in vitro* assay will yield results applicable to the *in vivo* situation. Therefore, wherever possible, *in vivo* studies should be used for the validation of *in vitro* models. *In vitro* methods have been developed to simulate the physiological conditions and the sequence of the events that occur during digestion in the human gastrointestinal tract (Cilla et al. 2018). In a first step, simulated gastrointestinal digestions applied to homogenized foods or isolated bioactive compounds in a closed system, with determination of the soluble component fraction obtained by centrifugation or dialysis of soluble components across a semi-permeable membrane (dialysis method) to obtain the bioaccessible fraction. Simulated gastrointestinal digestion can be performed with static models where the products of digestion remain largely immobile and do not mimic physical processes such as shear, mixing, hydration.

1.4 Food sources

According to Chun et al and IOM, Institute of Medicine, a report on Vitamin E has a Recommended Dietary Allowance (RDA) of 15 mg/day of α-T because is present in all products in highest quantity.

Vitamin E can be found in many foods like vegetables, fruit, plants and plants oils. The two predominant forms of tocopherols are α- and γ-, particularly researched for their effect on human health.

α-tocopherol can be found in almonds, avocados, hazelnuts, peanuts and sunflower seeds; β-tocopherol in oregano and poppy seeds; γ-tocopherol in pecans, pistachios, sesame seeds and walnuts; δ-tocopherol in edamame and raspberries. Common food oils including corn, peanut and soybean oil contain largely α-tocopherol and γ-tocopherol. Tocotrienols are bioconverted to tocopherols having many functions as intermediates in the biosynthesis of α-tocopherol in plants (Peh et al. 2016).

Fresh fruits and vegetables also contain amounts of tocopherols and tocotrienols, mostly with predominant concentrations of α- and γ-tocopherol. In general, α- and γ-tocotrienols content in fruits and vegetables are present at levels usually less than 0.1 mg/100 g (Chun et al. 2006), but these levels can change depending on the species or according to the heat treatment to which they go.

Tocotrienols are much less prevalent than tocopherols in fruits and vegetables, but they are mostly present in palm oil, barley, annatto and some cereal grains (Jiang 2014).

1.4.1 Cauliflower as source of Vitamin E

Cauliflower is a plant that belongs to Brassicaceae family. It is a plant cultivated since ancient times and over the centuries many varieties and cultivars have been selected.

Brassica varieties are divided into:

- Brassica oleracea var. acephala, to which belong the Tuscan black cabbace and the Galician cabbage
- Brassica oleracea var. alboglabra, the Chinese broccoli
- Brassica oleracea var. italica, the broccoli cabbage
- Brassica oleracea var. capitata, the cabbage used for the preparation of sauerkraut
- Brassica oleracea var. capitata rubra, the red cabbage
- Brassica oleracea var. costata or var. tronchuda, the Portuguese cabbage
- Brassica oleracea var. gemmifera, the Brussels cabbage

- Brassica oleracea var. gongylodes or L. var. caulorapa, the kohlrabi
- Brassica oleracea var. sabauda, the savoy cabbage
- Brassica oleracea var. sabellica, the kale
- Brassica oleracea var. ramosa
- Brassica oleracea var. botrytis (Figure 4)

Figure 4. Brassica oleracea var. botrytis. Green, white, orange and purple sample

According to FAOSTAT, the largest global producers of broccoli and cauliflower are China, India and the United States (average 1994-2017). Within the European Union, the major growers of broccoli and cauliflower are Spain, Italy and France (http://www.fao.org/faostat/en/#data/QC/visualize).

Cauliflower composition consists of glucosinolates, isothiocyanates, polyphenols, dietary fiber, proteins, tocopherols, carotenoids, minerals and other. Tocopherols in cauliflower are minor compounds if compared to glucosinolates and isothiocyanates, for which Brassica family is known, but they are relevant compounds for their nutritional value in a balanced diet (Campas-Baypoli et al., 2009).

Numerous epidemiological studies indicate that *Brassica* vegetables in general protect humans against cancer since they are rich sources of glucosinolates as well as possessing a high content of flavonoids, vitamins and mineral nutrients (Moreno et al. 2006). *Brassica* vegetables contain glucosinolates, the metabolic breakdown products of which are potent modulators of xenobiotic-metabolising enzymes that protect DNA from damage. This protective effect has been linked to the presence of glucosinolates in these vegetables. A high intake of cruciferous

vegetables is associated with a reduced risk of cancer, particularly lung and those of the gastrointestinal tract.

Recently, colored cauliflower has appeared on the market, being selected to become a stronger source of polyphenols and carotenoids when compared to white cauliflower. Violet one contains more polyphenols, while orange one is rich in carotenoids.

Table 1 reports the difference in tocopherol profile in white, cheddar and purple raw cauliflower.

Table 1. Values of tocopherols in raw whte, cheddar and purple cauliflower. Table taken from Guzman et al., 2012

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		Cauliflower	
Compound	White	Cheddar	Purple
α-T	61,5±6,3	65,8±0,9	84,5±10,0
γ-T	151,4±1	151,4±0,5	152,5±0,3
Total-T	212,9±7,2	217,2±1,5	236,6±10,3

Amounts are reported in μg/g DW ± standard deviation, where n = 3.

1.5 Heat Treatments in vegetables

Since prehistoric times, when about 2.5 billion of years ago the fire was discovered by Homo Erectus, there was an evolution of cooking methods leading to an improvement in chewing, digestibility and energy value.

Up to today many methods of cooking have been applied with different effects. The most common techniques used for vegetable consumption are sous-vide, boiling, steaming, microwave and blanching.

At the same time, cooking can have positive effects on foods but can cause also bioactive compounds losses, such as vitamins. Losses depend on cooking method and type of food. Degradation of vitamin depends on specific conditions during the process, e.g., temperature, presence of oxygen, light, moisture, pH, and duration of heat treatment. (Lešková et al. 2006)

1.5.1 Sous-vide

Sous-vide is a French term meaning "under vacuum" implicating that raw material or raw materials with intermediate foods are cooked under controlled conditions of temperature and time inside heat-stable vacuumed pouches (Baldwin 2012). Sous vide cooking differs from traditional cooking methods in two ways: the raw food is vacuum-sealed in heat-stable, food-grade plastic pouches and the food is cooked using controlled heating.

There are many potential benefits from this cooking method: it can be used to centralize food production and thus it reduce labour costs, extend shelf-life, improve nutrition and can give greater control of the cooking process to manipulate the behavior of food components creating new flavors and textures (Stringer and Metris 2018).

Sous vide cooked vegetables retain nearly all their nutritive value. It leaves the cell walls mostly intact and makes the vegetables tender by dissolving some of the cementing material that hold the cell together (Baldwin 2012).

According to Martìnez-hernandez et al (Martínez-hernández et al. 2013), there is scarce information reporting the effects of vacuum-based cooking methods on physical, sensory, microbial and nutritional quality of fruits and vegetables. It was not found differences in percentage folate retention after sous vide of broccoli. The highest vitamin C, B6 and folacin retentions (97–100 %) in broccoli cv. Shogun were achieved after sous vide, followed by steaming (83–100 %), and boiling (45–46 %). In a study, it was reported 60 % higher anthocyanin content for blue potato chips and 18, 19 and 51 % higher total carotenoids content for beans, mango chips and sweet potato chips, respectively, after vacuum deep-frying.

Furthermore, total Vitamin C decreased and total phenolics increased for higher vacuum-deep frying time and temperature for pineapple chips. The reported nutritional benefits after vacuum-based cooking procedures may be due to the low boiling temperature of water for vacuum boiling or oil for vacuum frying and to the low O2 cooking atmosphere, which induced the retention of the nutritional compounds and enhanced product colour. Sous vide allow the product to be cooked on its own juice, sealing in the flavour and aroma.

An important aspect to consider is the presence of pathogens that could survive during the cooking, such as *Salmonella, Listeria monocytogenes and Clostridium botulinum*. In fact foods prepared with this method should be consumed immediately after preparation or given a very limited shelf life to prevent multiplication of surviving pathogens.

1.5.2 Boiling

Boiling is the most common cooking method used up to now. It can be carried out in normal pots or in pressure cookers. A food can be boiled by immersing it in cold water and then bring it to the boiling point or immersing it in hot water.

According to Ramos dos Reis (Dos Reis et al. 2014), the boiling process have a detrimental effect on the content of polyphenols. Boiling induced an increase in the intensity of the colour in the plant.

As reported by Jimenez-Monreal et al (Jiménez-Monreal et al. 2009), in boiling occurs lixiviation phenomenon that leads to a 64% loss of total carotenoids and a 49% loss of total phenolics. The concentration of phenolic acids is highest in the outer layers of some vegetables and these are extremely exposed to the water, reducing antioxidant power of some vegetables such as spinach, cauliflower, and cabbage. However, total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing, individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the phenolic sugar glycosidic bounds. On the other hand, boiling may decrease the activity of ascorbic acid, while higher activity may occur as consequence of the inactivation of oxidative enzymes such as ascorbate oxidase. This fact reduces the browning potential and, although chlorogenic acid decreases, the ascorbic acid is retained for a longer time.

Generally, antioxidant concentrations and activities in processed vegetables were lower than those of corresponding raw sample. This was caused by their degradation, but also absorption of water during boiling, which diluted the compounds and decreased their content per weight unit (Podsedek 2007).

1.5.3 Steaming

Steaming is a method of cooking which use steam generated by boiling water continuously (Lafarga et al. 2018). This method uses can be applied by oven steaming or in special pots, one containing the water over which another pot is placed, dedicate to the food. Thanks to the cover, a circulation of air and steam is established in the pot thanks to which the food is cooked. The temperatures reached with this type of cooking about 100° C, boiling water temperature, slightly lower than traditional techniques, therefore it requires longer cooking times.

Even the technology has also done its part, first of all by making available special pots and then specialized steam ovens (Figure 5). In fact, it seems that with this cooking method there is a saving of time and fuel (Berretta M., 2017).

The foods cooked in this way maintain almost completely their nutrients, color, aroma, only the thermolabile vitamins (B1, B2 and C) are partly destroyed. From a nutritional point of view, cereals and legumes can be cooked in this way as the proteins become more assimilable with little relevant vitamin losses; while for fresh legumes and vegetables, for which the loss of vitamins and micro nutrients outweigh the advantages of quick preparation, it is less suitable (Berretta M., 2017).

Figure 5. Steam oven

1.5.4 Microwave

Usually, microwave is a cooking method used in domestic kitchens to heat or thaw foods. This is a technique used for its practicality and for the speed with which they act.

Microwave cooking is in fact one of the techniques that best preserves the nutritional values of food and enhances its natural aroma: in particular, fish and vegetables acquire a full flavor, more intense than any other type of cooking, except for sous vide.

Microwaves are electromagnetic radiations that heat the molecules of water, unable to transmit heat to the food. However, by causing water molecules oscillation, an intermolecular friction is generated, producing heat.

This is a cooking without surface browning, since the food normally does not exceed 100 ° C, a limit determined by the maximum temperature reached by the water at environmental pressure: in fact, the food begins to color quickly only when the water has completely evaporated (https://cucina.corriere.it/rubriche/scuola-di-cucina/25-febbraio-2010/tecnica-cottura-microonde_d050697e-221c-11df-8195-00144f02aabe.shtml?refresh_ce-cp).

In a study conducted by Jiménez-Monreal et al (Jiménez-Monreal et al. 2009), microwave heating retains the active components in the cooked tissue. The activity of vegetables cooked in themicrowave oven was generally higher than that of those cooked in boiling water, because microwave heating, griddling and baking does not stimulate the release of ascorbic acid or other antioxidants from cooked tissue. Allicin preserved its OH radical scavenging properties after microwaving.

1.5.5 Effect of cooking on tocopherol profile in cauliflower

In some cases, blanching or boiling a matrix like broccoli can increase vitamin E content through large losses of water-soluble components compared to the raw sample. In other types of heat treatments or vegetable matrix there may be a decrease of the amounts of Vitamin E contained within (Chun et al. 2006).

As reported by Anna Podsedek et al., (Podsedek 2007), the amounts of tocopherol and tocotrienols in Brassica vegetables are: broccoli (0.82 mg/100 g), Brussels sprouts (0.40 mg/100 g), cauliflower (0.35 mg/100 g), chinese cabbage (0.24 mg/100 g), red cabbage (0.05 mg/100 g) and white cabbage (0.04 mg/100 g). Other researchers have also reported similar rank on the basis of concentration, but in their study total tocopherol values were about 2-fold higher. These differences are probably caused by the differing varieties and growing conditions. It was reported, by these authors that kale was the best source of α-tocopherol and

γ-tocopherol (2.15 mg/100 g), but generally it was reported that α-tocopherol was predominant tocopherol in all Brassica vegetables, except in cauliflower, containing predominantly γ-tocopherol.

In contrast, other searches have reported lower concentration of γ-tocopherol than α-tocopherol in cauliflower. In general, the best sources of lipid-soluble antioxidants are kale and broccoli. Brussels sprouts have moderate levels of the above-mentioned compounds, while cauliflower and cabbage are characterized by their relatively low amounts.

According to Lee et al (Lee et al. 2018), in raw vegetables α-tocopherol was the major tocochromanol, in contrast, a low level of γ-tocopherol was determined in raw broccoli, mallow, crown daisy, perilla leaf, and zucchini. Levels are in agreement with other studies that show green leafy vegetables have higher vitamin E, occurring mainly as a-tocopherol and situated inside chloroplasts. Cooking vegetables lead to a significant increase in α-tocopherol. Green leafy or flower vegetables have a higher retention of α-tocopherol than root vegetables, which may be attributed to the increased extractability of α-tocopherol following denaturation of proteins and a complete breakdown of the cell wall in plants which occur as a result of cooking. In addition, there was a trend toward higher retention of c-tocopherol in cooked rather than raw vegetables.

This high content of vitamin E in cooked samples could be attributed to two reasons: the effect of heat treatment encountered during domestic cooking may cause softening of the tissue by cell disruption in plants and consequently result in the release of vitamin E from the lipids and then become more available for extraction or the heat treatment may also abolish the activity of tocopherol oxidase, which was found in all parts of plant like roots, stems, leaves, flowers and fruits. It has been suggested that oxidizing enzymes maybe involved in the loss of vitamin E during food processing. Plant tissue damage, caused by cutting or mixing could activate oxidizing enzymes involved in the loss of vitamin E due to the collapse of cell compartments, but heat treatment could deactivate endogenous oxidative enzymes (Lee et al. 2018).

Purpose of the thesis

The purpose of the thesis was to demonstrate the effect of cooking of various heat treatments on tocopherols profile, in a vegetable matrix such as cauliflower, orange and violet varieties. The heat treatments taken into consideration were sous-vide, steaming and boiled. Each of these was compared with the raw version of cauliflower.

2. Materials and method

2.1 Standards and reagents

α-tocopherol (>95%), γ-tocopherol (>95%), δ-tocopherol (>95%), ascorbic acid (>99.5%) were purchased from Sigma Aldrich (Milan, Italy) as well as all solvents HPLC grade used for standards, sample preparation and for liquid chromatography (hexane, isopropanol, acetic acid and ethanol). Sodium sulphate (99.0%) and potassium hydroxide (85%) were purchased from ITW Reagents (Milan, Italy). Ultrapure water was prepared using a milli-Q system (Millipore, Millford, MA, USA).

2.1.1 External standard

External calibration curves were used for quantitation of α-tocopherol, γ-tocopherol and δ-tocopherol as reported in Figure 6. α-tocopherol standard concentration ranged from 3.25 to 80.60 mg/L, γ-tocopherol from 3.25 to 97.50 mg/L and δ-tocopherol from 3.25 to 96.85 mg/L.

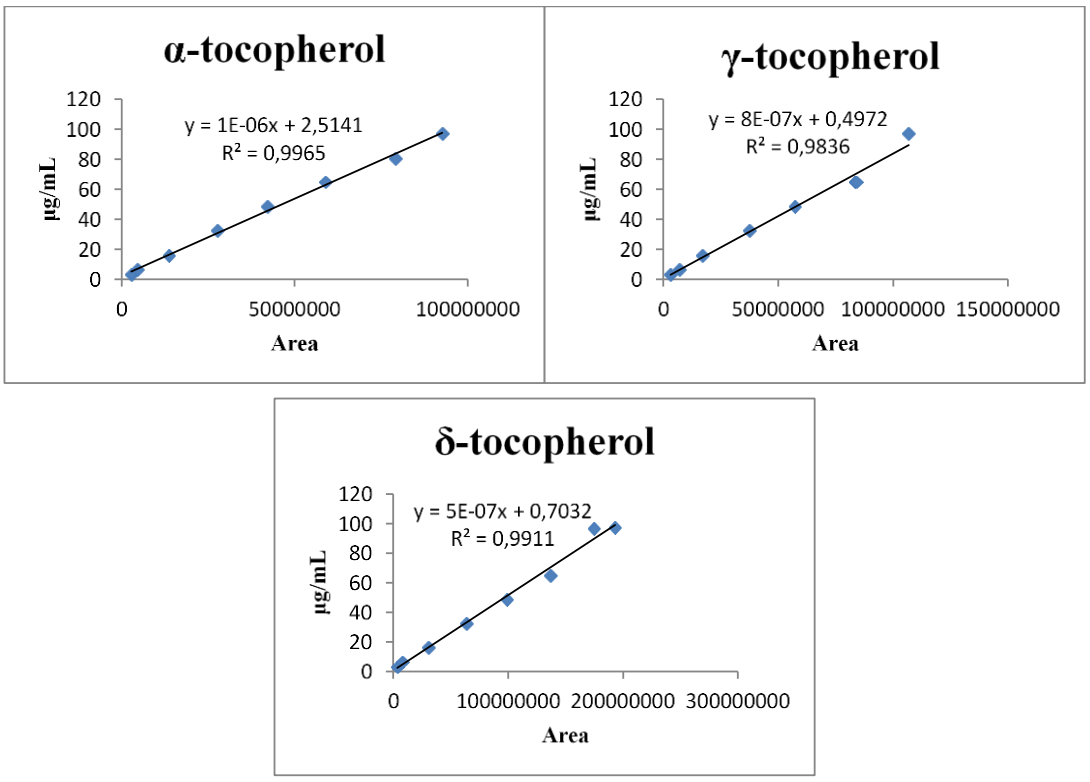

Figure 6. Calibration curves of α-, γ-, and δ-tocopherol

2.2 Samples

Brassica oleracea L. var. *botrytis*, "cheddar" (orange) and "depurple" (purple) were harvested in a local Italian company (Agrinovana, S.r.l., Petritoli, Fermo), cleaned removing the inedible parts. 6 kg of orange cauliflower were cut in pieces of 6-10 g and divided in 27 homogeneous portions (180-200g each). Three techniques of cooking were applied to cauliflower: boiling was performed placing cauliflower sample in 1.5 L of water at boiling point for 10 and 25 minutes; oven steaming was carried out using a glass container placed in the centre of an oven set up in full steam mode at 95°C, for 10, 25, 40 min; and sous-vide was performed in vacuum sealed bags placed in the centre an oven set up in traditional hot air mode at 95°C, for 10, 25 and 40 min. Each heat treatment, including raw samples, was carried out in triplicate as illustrated in Table 2, where the experimental design for violet and orange cauliflower is explained. After cooking, the samples were cooled down and milled for freeze-drying step. The samples were reduced to freeze-dried powder and kept under vacuum at -18°C (Figure 7).

Figure 7. Freeze-drying (VirTis, Wizard 2.0 Control System, Sp Industries, Gardiner, NY, USA)

Table 2. Sample of cauliflowers, types of cooking, cooking time, cooking temperatures.

Sample code	Type of cauliflower	Cooking	Time	Temperature
CVC	Violet	Raw	0	-
CVB10	Violet	Boiling	10	Point of boiling water
CVB25	Violet	Boiling	25	Point of boiling water
CVFS10	Violet	Steaming	10	95°C
CVFS25	Violet	Steaming	25	95°C
CVFS40	Violet	Steaming	40	95°C
CVSV10	Violet	Sous-vide	10	95°C
CVSV25	Violet	Sous-vide	25	95°C
CVSV40	Violet	Sous-vide	40	95°C
CAC	Orange	Raw	0	-
CAB10	Orange	Boiling	10	Point of boiling water
CAB25	Orange	Boiling	25	Point of boiling water
CAFS10	Orange	Steaming	10	95°C
CAFS25	Orange	Steaming	25	95°C
CAFS40	Orange	Steaming	40	95°C
CASV10	Orange	Sous-vide	10	95°C
CASV25	Orange	Sous-vide	25	95°C
CASV40	Orange	Sous-vide	40	95°C

2.2 Sample preparation

Saponification: 400 mg of freeze-dried sample was weighted in a centrifuge vial of 50 ml-screw top, added of 1 g of ascorbic acid, 0.1 g of sodium sulfate, 20 ml of ethanol, 4 ml of 80% KOH, shaked and placed in water bath at 85°C, for 30 min, shaking from time to time to prevent the sample from settling on the bottom of the vial.

Tocopherol extraction: after 30 min, the sample was cooled in ice for 10 minutes, added of 12 ml of water, and extracted with 20 ml of hexane, vortexed for 15 s and centrifuged for 2 min at 3600 rpm (Figure 8). The separated organic phase was transferred in a second vial of 50 ml. The procedure of extraction was repeated two more times: with 10 ml and 20 ml of hexane, pooling all the fraction in the second vial.

Washing: the pooled phase was washed with 10 ml of water, shaking sweetly to avoid emulsions, centrifuging for 2 min at 3600 rpm and eliminating the water from the bottom of the vial. This procedure was repeated for three more times. The washed *n*-hexane extracts were placed in a flask with a frosted neck of 100 ml and taken to dryness with rotavapor at 35°C, added of 1 ml hexane and transfer on a vial (2ml), centrifuged (3600 rpm, 2 minutes) and moving the clear hexane on a HPLC vial, ready for injection.

Figure 8. Centrifuge (Neya 16 R, Bench Top Centrifuges, Remi Elektrotechnik LTD, Mo – Italy)

2.3 UPLC analysis

UPLC was run on a Waters Ultra Pressure Liquid Chromatographic Acquity system (UPLC Acquity H-Class, Waters Corporation, Milford, CT, USA), Figure 9, equipped with a quaternary solvent manager, a sample manager, a column heater and a fluorimetric detector (FLD). The whole configuration was driven by Empower software v2.0 from Waters Corporation. An isocratic elution (8 minutes) of hexane with 0.4% isopropranol and 0.1% acetic acid at 0.3ml/min of flow. 2 min of re-equilibration between injections to avoid any carry-over effect. The extracts (2 μL) were loaded (30°C in the sampler) and separated in the direct phase column Ascentis Express Hilic (15cm x 2.1mm, 27 μm), at 30 °C. Tocopherols were identified by comparison of retention time with pure standards. Their quantification was performed by external calibration (see 2.1.1).

Figure 9 Uplc - Ultra Pressure Liquid Chromatographic

2.4 Statistical analysis

All analyses were carried out in three parallel replications and mean ± SDs were calculated for the values obtained. The data were analyzed by the analysis of variance (ANOVA), one way. It was used to check the significance of differences between mean values of raw and cooked material. The significance of differences was estimated with the Tukey test at the critical significance level of $p \leq 0.05$. The software used for statistical analysis was R-project.

3. Results and Discussion

3.1. Identification of tocopherols in cauliflower

Figure 10 reports a UPLC profile of tocopherols in orange and purple cauliflower. The compounds were recognized by comparing the retention times of pure standards with the sample. *α*-tocopherol, γ-tocopherol and *δ*-tocopherol were identified, thus for each compound a calibration curve was implemented to perform tocopherols quantification.

Figure 10a shows the peaks of tocopherols detected by UPLC/FLD in purple cauliflower. The compounds detected in larger quantities were γ-tocopherol followed by α-tocopherol. There is also a small amount of δ-tocopherol, although not quantifiable. Figure 10b shows the peaks of tocopherols detected by UPLC/FLD in orange cauliflower. The compounds detected in larger quantities were γ-tocopherol followed by α-tocopherol and δ-tocopherol.

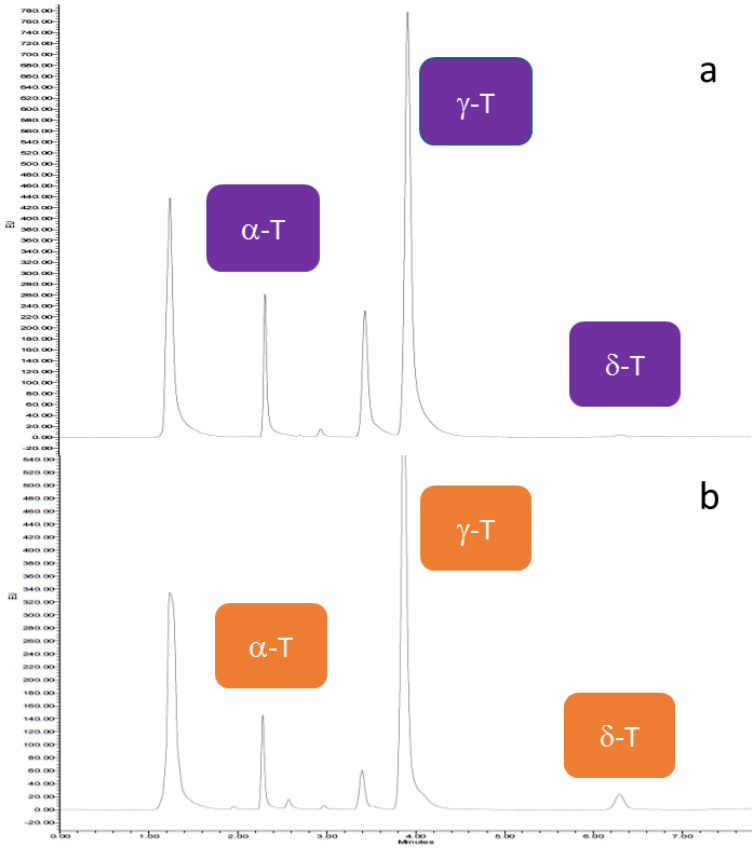

Figure 10. Example of UPLC/FLD profile of an orange and purple cauliflower sample showing the peaks of the identified tocopherols.

3.2. Quantitative analysis of tocopherols in orange and purple cauliflower

As result (Table 3), in all cauliflower samples (violet and orange, raw and cooked) the tocopherol revealed in highest concentration is the γ form, followed by the α form and then δ.

Table 3. In the table shows the results of the analysis. Are reported the name of the sample and values for α-tocopherol, γ-tocopherol, δ-tocopherol, total of tocopherols and the ratio between α-tocopherol and γ-tocopherol respectively for orange cauliflower (a) and for purple cauliflower (b). LOQ for alfa, gamma and delta tocopherols were 0.4, 0.3 and 0.4 mg/kg, respectively. Values in each column having different lowercase letters are significantly different at $p<0.05$

a)

sample	α-T	γ-T	δ-T	total	α-T/γ-T
CAC	8.4±0.6^{a}	17.9±3.8^{a}	2.2±0.1^{a}	28.5±4.4^{a}	0.47±0.07^{b}
CAB10	20.4±2.3^{d}	106.7±17.7^{c}	4.8±0.5^{cd}	131.8±19.9^{c}	0.19±0.01^{a}
CAB25	26.6±2.8^{e}	150.7±20.6^{d}	6.1±0.9^{d}	183.4±24.1^{d}	0.18±0.00^{a}
CAFS10	14.5±1.6^{bc}	66.9±12.0^{b}	3.4±0.5^{ab}	84.8±13.8^{b}	0.22±0.02^{a}
CAFS25	15.7±2.2^{cd}	80.4±14.5^{bc}	3.7±0.4^{bc}	99.7±16.5^{bc}	0.19±0.01^{a}
CAFS40	14.4±0.7^{bc}	68.9±8.6^{b}	3.5±0.3^{ac}	86.8±9.4^{b}	0.21±0.01^{a}
CASV10	10.1±2.1^{ab}	46.4±11.5^{ab}	3.4±0.4^{ab}	59.8±10.6^{ab}	0.22±0.07^{a}
CASV25	14.7±1.4^{bc}	65.2±8.4^{b}	3.3±0.5^{ab}	83.3±10.2^{b}	0.23±0.01^{a}
CASV40	14.4±0.3^{bc}	63.1±4.4^{b}	3.5±0.6^{ac}	81.0±4.7^{b}	0.23±0.01^{a}

b)

sample	α-T	γ-T	δ-T	total	α-T/γ-T
CVC	11.8±2.0^{a}	26.0±9.3^{a}	<LOD	37.8±11.3^{a}	0.47±0.09^{b}
CVB10	20.8±1.3^{c}	66.3±8.3^{b}	<LOQ	87.1±9.5^{b}	0.44±0.02^{ab}
CVB25	29.7±2.8^{d}	104.5±7.5^{c}	<LOQ	134.1±10.2^{c}	0.39±0.01^{a}
CVFS10	17.6±2.1^{bc}	46.9±10.7^{ab}	<LOD	64.5±12.6^{ab}	0.32±0.06^{ab}
CVFS25	15.0±1.0^{ab}	36.3±6.4^{a}	<LOD	51.4±7.3^{a}	0.31±0.05^{ab}
CVFS40	15.8±2.6^{ac}	40.6±7.8^{a}	<LOD	56.3±10.3^{a}	0.31±0.02^{ab}
CVSV10	12.8±1.8^{ab}	27.8±7.7^{a}	<LOD	40.6±8.9^{a}	0.28±0.11^{b}
CVSV25	14.5±0.6^{ab}	34.1±3.7^{a}	<LOD	48.5±3.9^{a}	0.30±0.05^{ab}
CVSV40	15.6±2.1^{ac}	36.2±5.7^{a}	<LOD	51.8±7.8^{a}	0.35±0.02^{ab}

Considering the raw samples, tocopherol levels changed according the cauliflower variety: in orange sample, α-tocopherol showed a value of 8.4±0.6 mg/kg, γ-tocopherol a value of 17.9±3.8 mg/kg and δ-tocopherol a value of 2.2±0.1 mg/kg. While, in purple raw sample, α-tocopherol showed a value of 11.8±2.0 mg/kg and γ-tocopherol a value of 26.0±9.3 mg/kg. According to Guzman et al (2012), δ-tocopherol was not present in detectable quantities, both for orange and violet variety.

Considering the cooked samples, all heat treatments lead to an increase of tocopherols levels. It is supposed that the temperature degrades the molecular structure of cauliflower, facilitating the release of tocopherols compounds. In cooked orange cauliflower, α-tocopherol value ranged from 10.1±2.1 to 26.6±2.8 mg/kg, γ-tocopherol value ranged from 46.4±11.5 to 150.7±20.6 mg/kg and δ-tocopherol value ranged from 3.3±0.5 to 6.1±0.9 mg/kg. While for cooked purple cauliflower the value for α- and γ-tocopherol ranged respectively from 12.8±1.8 to 29.7±2.8 mg/kg and 27.8±7.7 to 104.5±7.5 mg/kg. As for the raw sample, in cooked sample δ-tocopherol was not present in detectable quantities. This high content of tocopherols in cooked samples could be attributed to two reasons: the effect of heat treatment encountered during domestic cooking may cause softening of the tissue by cell disruption in plants and consequently result in the release of vitamin E from the lipids and then become more available for extraction and the heat treatment may also abolish the activity of tocopherol oxidase, which was found in all parts of plant like roots, stems, leaves, flowers and fruits (Lee et al. 2018).

3.2.1 Effect of time of heating on boiling treatment

As showed in the Figure 11, boiling (blue line) presents only two heating time, 10 and 25 minutes. The highest levels of all tocopherols were obtained after 25 minutes of heating. However, after 10 minutes large quantities of tocopherols were reached until the maximum at 25 minutes. According to Mazzeo et al., and Girgin et al., (Mazzeo et al. 2011; Botrytis and El 2015), during boiling, the water soluble compounds can leach into boiling water. These losses are greatest in the case of leafy or highly fragmented vegetables. So boiling presents positive and negative effect on cauliflower, but generally it depends on vegetable matrix.

3.2.2 Effect of time of heating on sous vide

Sous vide treatment (green line) presents three different heating time: 10, 25 and 40 minutes. From the graphics of Figure 11 it can be noticed a positive increase of all tocopherols between the three types of cooking times, but not statistically significant for 25 and 40 minutes. 10 minutes are enough to reach the maximum levels of all tocopherols which however are not different from raw sample levels for both cauliflower varieties. Only the orange one at 25 and 40 min showed little higher incrementation of all tocopherols than raw samples.

Our results are in disagreement with Dos Reis et al., (Dos Reis et al. 2014) since their sous vide processing resulted in greater antioxidant capacity than boiling, steaming and microwaving in both cauliflower and broccoli. However, they did not consider tocopherols for the evaluation of antioxidant capacity.

3.2.3 Effect of time of heating on steaming oven

Reported in Figure 11, steaming treatment (red line) presents three heating time: 10, 25 and 40 minutes. In purple cauliflower, no significant differences were revealed among the γ-tocopherol values in samples obtained at different cooking times. It is enough 10 min of cooking to reach the maximum levels α and γ-tocopherols. The same trend is showed for orange cauliflower.

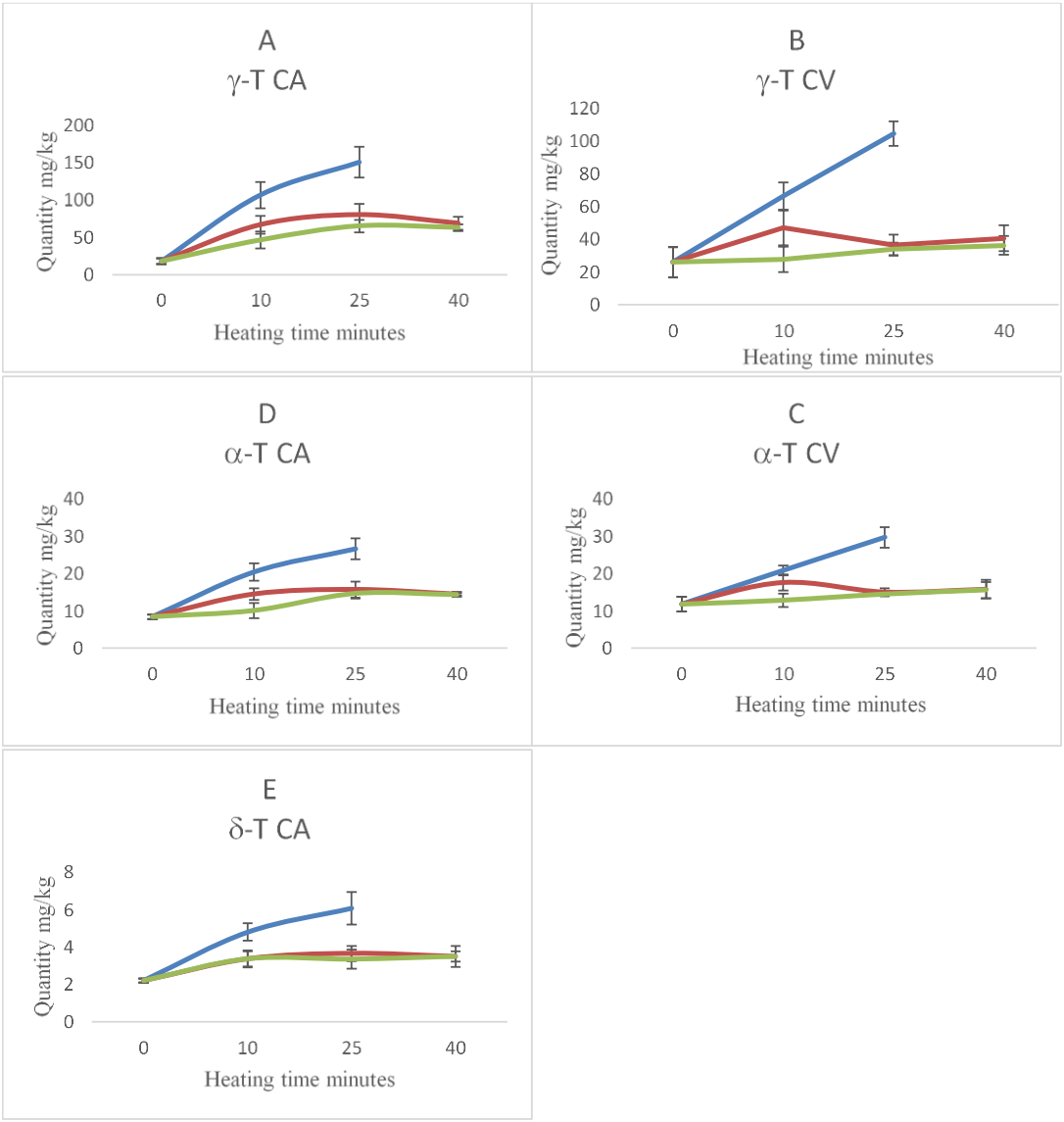

Figure 11. Evolution of α-tocopherol, γ-tocopherol and δ-tocopherol (mg/kg dried) in orange (CA) and violet (CV) cauliflower cooked with three different techniques: boiling (blue), steaming (red), sous vide (green) for different times of heating (0,10,25,40). Values are means of three replicates ± standard deviation.

4. Conclusions

Orange and purple cauliflower showed different tocopherol profile as orange one contained also δ- tocopherol, not only α and γ form.

The temperature of thermal process (95°C) had a positive influence on the extractability of tocopherols, confirming that the degradation of bioactive compounds in vegetables is matrix dependent.

As the purpose of this study was to verify which cooking technique in terms of highest concentration of tocopherols in a vegetable matrix such as cauliflower, it emerged that the best cooking technique is boiling with 131.8±19.9 mg/kg and 183.4±24 mg/Kg for the orange one and 87.1±9.5 mg/kg and 134.1±10.2 mg/kg for the purple one.

Steaming and sous vide techniques at the times of 25 and 40 seem to have the same effect in terms of extraction, showing smaller amount of tocopherols in all three forms α-, γ- and δ- than boiling. Steaming and sous vide consist in a non-direct contact with water, which did not enhance the extraction of bioactive compounds such as tocopherols.

This study provides information on traditional and innovative cooking methods and their effect on tocopherols, compounds which are less investigated in a matrix such as cauliflower.

5. 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.

Berretta M., D. F. 2017. *NutriAmo la Vita - suggerimenti per un corretto stile di vita, prima, durante e dopo la malattia tumorale.* Verduci Editore.

Botrytis, L, and Sedef Nehir El. 2015. "Journal of Food Composition and Analysis Effects of Cooking on in Vitro Sinigrin Bioaccessibility , Total Phenols , Antioxidant and Antimutagenic Activity of Cauliflower" 37: 119–27. https://doi.org/10.1016/j.jfca.2014.04.013.

Chun, Jiyeon, Junsoo Lee, Lin Ye, Jacob Exler, and Ronald R. Eitenmiller. 2006. "Tocopherol and Tocotrienol Contents of Raw and Processed Fruits and Vegetables in the United States Diet." *Journal of Food Composition and Analysis* 19 (2–3): 196–204. https://doi.org/10.1016/j.jfca.2005.08.001.

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.

Jiang, Qing. 2014. "Natural Forms of Vitamin E: Metabolism, Antioxidant, and Anti-Inflammatory Activities and Their Role in Disease Prevention and Therapy." *Free Radical Biology and Medicine* 72: 76–90. https://doi.org/10.1016/j.freeradbiomed.2014.03.035.

Jiménez-Monreal, A. M., L. García-Diz, M. Martínez-Tomé, M. Mariscal, and M. A. Murcia. 2009. "Influence of Cooking Methods on Antioxidant Activity of Vegetables." *Journal of Food Science* 74 (3). https://doi.org/10.1111/j.1750-3841.2009.01091.x.

Lafarga, Tomás, Gloria Bobo, Inmaculada Viñas, Lorena Zudaire, Joan Simó, and Ingrid Aguiló-aguayo. 2018. "International Journal of Gastronomy and Food Science Steaming and Sous-Vide : E Ff Ects on Antioxidant Activity , Vitamin C , and Total Phenolic Content of Brassica Vegetables." *International Journal of Gastronomy and Food Science* 13 (January): 134–39. https://doi.org/10.1016/j.ijgfs.2018.05.007.

Lee, Seongeung, Youngmin Choi, Heon Sang Jeong, Junsoo Lee, and Jeehye Sung. 2018. "Effect of Different Cooking Methods on the Content of Vitamins and True Retention in Selected Vegetables." *Food Science and Biotechnology* 27 (2): 333–42. https://doi.org/10.1007/s10068-017-0281-1.

Lešková, Emília, Jana Kubíková, Eva Kováčiková, Martina Košická, Janka Porubská, and Kristína Holčíková. 2006. "Vitamin Losses: Retention during Heat Treatment and Continual Changes Expressed by Mathematical Models." *Journal of Food Composition and Analysis* 19 (4): 252–76. https://doi.org/10.1016/j.jfca.2005.04.014.

Martínez-hernández, Ginés B, Perla A Gómez, Presentación García-gómez, and Francisco Artés. 2013. "Innovative Cooking Techniques for Improving the Overall Quality of a Kailan-Hybrid Broccoli," 2135–49. https://doi.org/10.1007/s11947-012-0871-0.

Mazzeo, Teresa, Denis N Dri, Emma Chiavaro, Attilio Visconti, Vincenzo Fogliano, and Nicoletta Pellegrini. 2011. "Effect of Two Cooking Procedures on Phytochemical Compounds , Total Antioxidant Capacity and Colour of Selected Frozen Vegetables." *Food Chemistry* 128 (3): 627–33. https://doi.org/10.1016/j.foodchem.2011.03.070.

Moreno, D. A., M. Carvajal, C. López-Berenguer, and C. García-Viguera. 2006. "Chemical and Biological Characterisation of Nutraceutical Compounds of Broccoli." *Journal of Pharmaceutical and Biomedical Analysis* 41 (5): 1508–22. https://doi.org/10.1016/j.jpba.2006.04.003.

Nesaretnam, Kalanithi. 2008. "Multitargeted Therapy of Cancer by Tocotrienols." *Cancer Letters* 269 (2): 388–95. https://doi.org/10.1016/j.canlet.2008.03.063.

Peh, Hong Yong, W. S.Daniel Tan, Wupeng Liao, and W. S.Fred Wong. 2016. "Vitamin E Therapy beyond Cancer: Tocopherol versus Tocotrienol." *Pharmacology and Therapeutics* 162: 152–69. https://doi.org/10.1016/j.pharmthera.2015.12.003 Associate Editor: Y. Zhang.

Podsedek, Anna. 2007. "Natural Antioxidants and Antioxidant Capacity of Brassica Vegetables: A Review." *LWT - Food Science and Technology* 40 (1): 1–11. https://doi.org/10.1016/j.lwt.2005.07.023.

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.

Stringer, Sandra Caroline, and Aline Metris. 2018. "Predicting Bacterial Behaviour in Sous Vide Food." *International Journal of Gastronomy and Food Science* 13 (September 2017): 117–28. https://doi.org/10.1016/j.ijgfs.2017.09.001.

Ulatowski, L, R Parker, G Warrier, R Sultana, D A Butterfield, and D Manor. 2014. "VITAMIN E IS ESSENTIAL FOR PURKINJE NEURON INTEGRITY." *Neuroscience* 260: 120–29. https://doi.org/10.1016/j.neuroscience.2013.12.001.