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Influence of the freezing process on the functional rheological properties of homogenised vegetable products

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Abstract

Achieving adequate storage, preservation, and export of vegetable products while maintaining their rheological properties and nutritional benefits remains a persistent challenge. Freezing, as one of the best and most natural methods for preserving taste and nutrition, is the most common procedure for achieving this goal. It acts as a natural preservative by slowing bacterial growth and preventing spoilage and oxidation by turning residual moisture into ice without a meaningful alteration in the nutritional content. Additionally, freezing significantly reduces chemical and enzymatic activity. It impacts bioactive molecules, such as antioxidants, vital in combating free radicals responsible for cell damage and ageing**.** Measure of antioxidant activity by standard methods for different varieties allows us to find the best situation for preservation.

In this thesis, the antioxidant activity was assessed before and three months after freezing by the ABTS method (to evaluate the ability of antioxidants to scavenge the ABTS radical cation), the Folin-Ciocalteu method, for determining the total phenolic content which is related to antioxidant capacity and DPPH assay to measure the capacity of an antioxidant to donate hydrogen atoms to neutralise the DPPH radical which are stable free radicals that change colour when exposed to antioxidants.

Analysis of four culinary products, beetroot, cauliflower, parsley, and broccoli, before and after three months of freezing demonstrated a significant effect on both the percentage of antioxidant activity (AOA) and total phenolic content (TPC), particularly in certain varieties. In contrast, the minimal changes observed in other varieties highlight that resistance to freezing-induced stress varies among cultivars.

In conclusion, freezing can influence the nutritional and functional qualities of stored produce. Notably, certain varieties exhibited a significant reduction in these parameters, whereas others maintained their AOA and TPC levels more consistently. This variation underscores the importance of cultivar-specific responses to freezing-induced stress, suggesting that some cultivars have an innate resilience that makes them more suitable for extended freezing.

Riassunto

Laa conservazione, lo stoccaggio dei prodotti vegetali, mantenendone le proprietà reologiche e i benefici nutrizionali, sono una sfida. Il congelamento, uno dei metodi più naturali ed efficaci per preservare gusto e valore nutrizionale, è la procedura più comune per raggiungere questo obiettivo. Agisce come conservante naturale rallentando la crescita batterica e prevenendo il deterioramento e l'ossidazione trasformando làcqua in ghiaccio senza alterazioni significative del contenuto nutrizionale. Inoltre, il congelamento reduce significativamente le reazioni chimiche ed enzimatichea. Ha tuttavia un impatto su molecole bioattive, come gli antiossidanti, essenziali nel combattere i radicali liberi responsabili dei danni cellulari e dell'invecchiamento. La misurazione dell'attività antiossidante mediante metodi standard per diverse varietà consente di identificare le condizioni ottimali per la conservazione.

In questo studio, si e'determinata l'attività antiossidante prima e tre mesi dopo il congelamento, utilizzando metodi come l'ABTS per valutare la capacità degli antiossidanti di neutralizzare il catione radicale ABTS, il Folin-Ciocalteu per determinare il contenuto fenolico totale, correlato alla capacità antiossidante attraverso una reazione redox, e il test DPPH per misurare la capacità di un antiossidante di donare atomi di idrogeno per neutralizzare il radicale DPPH, stabile e che cambia colore quando esposto agli antiossidanti.

L'analisi di quattro prodotti vegetali barbabietola, cavolfiore, prezzemolo e broccoli—prima e dopo tre mesi di congelamento ha dimostrato un effetto significativo del congelamento sia sulla percentuale di attività antiossidante (AOA) sia sul contenuto fenolico totale (TPC), in particolare in alcune varietà. Al contrario, i cambiamenti minimi osservati in altre varietà evidenziano che la resistenza allo stress indotto dal congelamento varia tra i cultivar.

Il congelamento può influenzare le qualità nutrizionali e funzionali dei prodotti conservati. In particolare, alcune varietà hanno mostrato una riduzione significativa di questi parametri, mentre altre hanno mantenuto più costantemente i livelli di AOA e TPC. Questa variazione evidenzia l'importanza delle risposte specifiche dei cultivar allo stress indotto dal congelamento, suggerendo che alcune cultivar posseggano una resilienza innata che li rende più adatti a lunghi periodi di congelamento.

1. Introduction

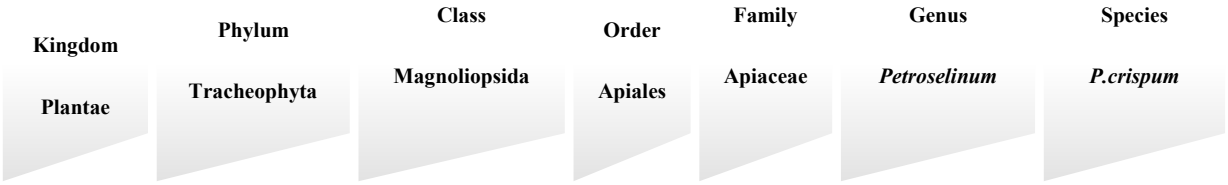
Advances in food preservation technology have played a pivotal role in ensuring the sustenance of the world's growing population (Năstase et al., 2016). In promoting a balanced diet, vegetables are highly recommended. Their consumption is not only beneficial for health but also contributes to cost savings in healthcare. The absence of vegetables in one's daily diet can lead to increased overall health service expenses (Del Pozo-Rubio et al., 2018; Knez et al., 2022; Melbye et al., 2021). In today's food landscape, consumers are increasingly focused on the quality and safety of their food. However, they also place significant importance on the nutritional value and sensory attributes of their chosen products (CÄ‚Tunescu et al., 2012). The beneficial effects of consuming plant-based foods are often attributed to the presence of bioactive molecules, including antioxidants (Gey, 1986; Neri et al., 2020; Rice-Evans & Miller, 1995) . Culinary herbs have the potential to be a significant source of antioxidants in the diet (Chohan et al., 2008). Free radicals are unstable molecules that can damage cells. They form naturally in the body through activities conducted in mitochondria and endoplasmic reticulum, as well as from outside factors like pollution, tobacco, and certain drugs. When free radicals build up, they harm DNA, proteins and fats, disrupting cellular balance and causing oxidative stress, which is associated with aging and disease (Phaniendra et al., 2014). Antioxidants are compounds that help protect the body's cells from oxidative damage caused by free radicals, which are unstable molecules that can cause cell damage and contribute to various chronic diseases and death (Fridovich, 1999; H. Zhang et al., 2006). Numerous studies have consistently demonstrated that the presence of natural antioxidants found in various medicinal plants and aromatic plants is strongly associated with a reduction in the risk of chronic diseases. These include conditions characterized by carcinogenesis, DNA damage and mutagenesis (Covacci et al., 2001; Craig, 1999; H. Zhang et al., 2006). Brain cells, because of their large oxygen consumption, are one of the parts most sensitive to oxidative stress (Haces et al., 2010; Vranješ et al., 2021). Furthermore, the impact of free radicals on the brain can result in severe neurological conditions, including dementia, stroke, and Alzheimer's disease (Bains & Hall, 2012; Vranješ et al., 2021) .Following traumatic brain injuries, there is an

escalation in the production of reactive species and lipid peroxidation processes, which can subsequently lead to extensive damage and, in some cases, fatal outcomes (Vranješ et al., 2021). The primary wellspring of natural antioxidants largely stems from food derived from plants and, to a lesser extent, animal-derived food products. Plant-based antioxidants are predominantly characterised by polyphenols, encompassing phenolic acids, a diverse range of flavonoids, including tannins and anthocyanins, along with vitamins A, C, tocopherols, organic acids, carotenoids, selenium, and calcium (Kobus-Cisowska et al., 2010). The antioxidant potential and polyphenol content in raw vegetables may not necessarily surpass that of their processed counterparts, as factors such as elevated temperature and other variables can enhance the greater bioavailability of the active components. This suggests that the methods of preparation and cooking can influence the nutritional value of vegetables and that higher temperatures can unlock more excellent benefits from these foods, challenging the assumption that raw vegetables always hold the highest levels of these health-promoting compounds (Dos Reis et al., 2015; Kosewski et al., 2023). Freezing is a method for preserving the quality and extending the shelf life of various products that involves lowering the temperature of a product to below its freezing point to avoid food spoilage, for centuries. This inhibits not only the growth of microorganisms but also chemical and enzymatic reactions. Therefore, spoilage is prevented, the product's shelf life is extended, and freshness and flavour are preserved. For long-term food storage, a temperature below −18 °C is recommended. Freezing can also affect the functional and rheological properties of these products, ultimately impacting their texture, taste, and overall quality (Năstase et al., 2016; Tressler & Evers, 1946). In this thesis, the influence of freezing on the functional and rheological properties of five culinary plants will be explored. Specifically, parsley, beetroot, broccoli, cauliflower, and carrots will be considered.

1.1 Taxonomic hierarchy of vegetables used in this work: parsley, beetroot, broccoli, cauliflower, and carrot

1.1.1 Taxonomic hierarchy of the genus *Petroselinum crispum* (Parsley)

According to the U.S. DEPARTMENT OF AGRICULTURE the taxonomic for Parsley can be represented in the following chart (USDA Plants Database Classification ID Report)



Petroselinum sativum Hoffm, from Family Umbelliferae, is commonly recognized as "parsley". It's worth noting that *Apium crispum* Mill is a synonymous term for *Petroselinum sativum* Hoffm. (Agyare et al.,2017; Es-Safi et al., 2021) The Umbelliflorae order includes seven families, and the most abundant is the Apiaceae family, which counts 275 genera and 2850 species growing around the world (Živković et al., 2021). This plant family is frequently called the Apiaceae family due to its distinctive umbrella-shaped inflorescences (Živković et al., 2021). Parsley comes in two primary cultivars: one with curly leaves and another with flat leaves. This herb produces an umbrella-like cluster of tiny flowers and distinctively ribbed seeds (*Parsley Uses, Benefits & Side Effects - Drugs.Com Herbal Database*, 2023)

1.1.2 Taxonomic hierarchy of genus of *Beta vulgaris* (Beetroot)

The botanical description of *Beta vulgaris* is represented in the following chart: (Pandita et al., 2020)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheobionta	Magnoliopsida	Caryophyllales	Chenopodiaceae	*Beta*	*B.vulgaris*

One classification system for cultivated plants for Beetroot classifies it into four different cultivar groups: Fodder Beet, Garden Beet, Sugar Beet, and Leaf Beet (Abd El-Wahab et al., 2022; Lange et al., 1999).

The Chenopodiaceae is one of the important botanical families and, in terms of its taxonomy and diagnostics, is one of the most challenging groups of flowering plants (Sukhorukov et al., 2019)

1.1.3 Taxonomic hierarchy of genus of *Brassica oleracea* Var. *Italica* (Broccoli)

According to USDA, the classification of Broccoli (*Taxon: Brassica Oleracea Var. Algoglabra x Brassica Oleracea Var. Italica,* n.d.)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheophytes	Magnoliopsida	Brassicales	Brassicaceae	*Brassica*	*B.oleracea*

A practical distinction between cauliflower and broccoli can be observed in their ontogeny, particularly at the mature and harvest stages, aligning with their taxonomic classification. Cauliflower forms a dense, white curd composed of undeveloped flower buds, while broccoli produces green florets with distinct flower clusters on branching stalks. This developmental difference reflects their classification within the Brassicaceae family, where each has evolved specific traits that make them easily distinguishable at maturity (Gray, 1982)

1.1.4 Taxonomic hierarchy of genus of *Brassica oleracea* Var. *botrytis* (Cauliflower)

According to USDA, the classification of Cauliflower (*Taxon: Brassica Oleracea Var. Algoglabra x Brassica Oleracea Var. Italica*, n.d.)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheophytes	Magnoliopsida	Brassicales	Brassicaceae	*Brassica*	*B.oleracea*

Most varieties of Botrytis cauliflower are characterised by white or cream-coloured curds. However, forms with coloured curds also exist, where pigments are distributed throughout the curd tissue. Additionally, occasional colour variants can sometimes emerge within populations of typically white-curded varieties, adding to the diversity of cauliflower forms (Crisp et al., 1975; Gray, 1982)

1.1.5 Taxonomic hierarchy of genus of *Daucus carota* subsp. *Sativus* (Carrot)

According to USDA, the classification of carrots (*Taxon: Daucus Carota Ssp. Sativus*, n.d.)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheophytes	Magnoliopsida	Apiales	Apiaceae	*Daucus*	*D.Carota*

1.2 Growing and planting vegetables used in this work

1.2.1 Growing and planting parsley

Parsley is an herb with extensive cultivation and usage. Its origins trace back to the Mediterranean region, encompassing Spain, Morocco, Malta, Algeria, Italy, Tunisia, and Greece but it has found its way into gardens across the globe (Craft et al., 2017) This healing plant boasts a rich history spanning over two millennia in Mediterranean cultures. Initially employed by the Ancient Greeks in religious ceremonies, it later gained fame as a diuretic, thanks to Hippocrates. The Romans introduced parsley to Central Europe, and by 795 CE, its cultivation became a legal obligation under the rule of Charlemagne (Živković et al., 2021). Now a day, parsley is widely employed as a spice and is grown across the globe. It grows in Mediterranean climates or warm temperate. The ideal temperature for its germination is around 20 ºC. Parsley is a shallow-rooted plant that demands consistent soil moisture levels. Insufficient water supply can impede leaf growth and limit overall yields (Petropoulos et al., 2008). It thrives in well-drained, nutrient-rich soils with a substantial organic matter content so long daylight hours and full sunlight is the ideal for its growth. Parsley is not well-suited to drought conditions due to its delicate plant tissue. The plant performs best when cultivated in soils with an optimal pH of 6.5. Parsley boasts deep roots and has a significant demand for water (Islam et al., 2004; Mylavarapu & Zinati, 2009; Petropoulos et al., 2008; Sarwar et al., 2016). Unfortunately, Parsely, like other plants, has some infectious diseases. A report from Iran highlights a plant disease known as "Yellow Dwarf Disease" that has become a significant issue for parsley farms. In December 2018, *Petroselinum crispum*, cultivated in the Kerman province of Iran, displayed distinct symptoms. These symptoms included the stunting of plant growth and a widespread yellowing of the plant's leaves. In a study on parsley growth, using beneficial microbes like *Streptomyces fulvissimus* AtB-42 and *Trichoderma harzianum* T22 had a positive impact. After three months, plants treated with these single cultures showed increased height compared to the control group. Additionally, all treatment groups had higher fresh weight compared to the control group, indicating improved growth and development in parsley with microbial treatments (Staropoli et al., 2021).

Further investigation and research conducted on the affected parsley plants revealed the presence of a type of virus called “nanovirus" (Figure1). This virus was identified as one of the contributing factors to the Yellow Dwarf Disease complex in the Kerman province, indicating that the disease is likely caused by a combination of various factors, with nanovirus being one of them. It marks the first documented evidence of this virus, referred to as "PSSaV," infecting parsley in Iran (Hasanvand et al., 2020; Vetten et al., 2019)

Subjecting parsley plants to elevated levels of carbon dioxide (627 ± 24 μmole CO^2 mole−1 air) led to a notable enhancement in biomass production, indicating that the parsley plants thrived and produced more plant material under these conditions. The study also delved into the composition of the plants by assessing the accumulation of both soluble and insoluble sugars, as well as organic acids. Under the elevated CO_2 treatment, all sugar fractions showed increased accumulation, highlighting a positive impact on the plant's carbohydrate content. Specifically, citric and isobutyric acids exhibited significant increases, suggesting a distinct response to elevated carbon dioxide. In contrast, malic acids and fumaric did not show significant changes, indicating that their levels remained relatively stable under these conditions. Notably, the experiment revealed a significant improvement in the levels of oxalic acids and succinic, signifying a specific enhancement in producing these organic acids in response to elevated CO_2 (Saleh et al., 2018)

Figure 1: The effect of Nanovirus on Parsley

1.2.2 Growing and planting of beetroot

In moderate climates, Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is one of the essential crops which provides approximately 30% of the world's annual sugar production and is used as animal feed and also a feedstock for producing bioethanol (Dohm et al., 2014).

Sugar beet is one of the most recently domesticated crops: in the late eighteenth century, lines accumulating sugar in the storage root were selected from crosses made with chard and fodder beet; on the other hand, Leafy beets have been cultivated since Roman times (Dohm et al., 2014; Monteiro et al., 2013).

Sucrose is more than 98% of the total root sugar, while glucose and fructose are present in very tiny amounts (Turesson et al., 2014; Varga et al., 2021).

For sugar beet root growth and sugar yield, the leaf area is crucial. In spring-sown sugar beets, the leaf area peaks in late July to early August, then gradually declines as the season ends. The ideal leaf area index (LAI) for most crops, including sugar beet, is around 3–4 m^2/m^2, maximising photosynthesis and yield (Roslon et al., 2005; Varga et al., 2021). In another investigation, it was found that the optimum LAI for sugar beet was from mid-June till the end of July (Varga et al., 2021).

1.2.3 Growing and planting broccoli

Broccoli is widely produced in many American and European countries. The American Cancer Society considers it a highly nutritious and anti-cancerous food. The optimum temperature of broccoli growth is the range between 18-24 ˚C. The growth process is time-dependent and often follows multiplicative patterns, making them easier to describe using relative rates, such as relative growth rate or net assimilation rate. These rates provide a clear understanding of growth progress over time (Getachew et al., 2016).

The appropriate date of broccoli planting is essential for achieving maximum yield and profitability. If the plant is exposed to unsuitable weather conditions, it will remain in the vegetative phase and fail to develop a head. besides this, weather conditions,

especially after floral initiation, can play a critical role in determining head quality. Key factors that influence curd initiation and the overall quality of the head include the plant's physiological age, the number of leaves before curd formation, and the temperature environment during growth. Properly managing these factors is crucial for successful broccoli production (Getachew et al., 2016).

For better growth and maximise yield, it requires large amounts of nitrogen (Greenwood et al., 2005). The amount of nitrogen rates ranges from 112 to 450 kg N ha^{-1} for optimum broccoli yields (Bakker et al., 2011).

1.2.4 **Growing and planting cauliflower**

Cauliflower (*Brassica oleracea* var. *botrytis*), belonging to the family Brassicaceae, is one of the most globally popular vegetable crops cultivated in many regions in the world. The optimum temperature for growth has been established between 15 ˚C and 20 ˚C. Because of the weak root systems, soil with rich humus is crucial, and it tends to soil with a pH between 6.5 and 7.5 which is vital for the best development (Sultana et al., 2019).

1.2.5 **Growing and planting carrot**

Among the top ten most economically important vegetable crops in the world in terms of both area of production and market value, carrot (*Daucus carota L.*) is significant. For increased yield, planting from November 15th to December is the best time; some authors reported that late planting resulted in low yield and increased susceptibility to disease (Malek et al., 2012; Shamsul Alam et al., 2020)

1.3 Botanical characteristics of vegetables used in this work

1.3.1 Botanical characteristics of parsley

Parsley is a plant with aromatic qualities that is classified as a biennial and belongs to the Apiaceae family, also known as the Carrot family. This plant is characterized by specific botanical features, including an unbranched root system, leaves that are divided in a feather-like or pinnate pattern, umbrella-like clusters of small flowers called "umbels," and dry, split fruits known as "schizocarps" (Živković et al., 2021).

Parsley has been grown in temperate and subtropical regions worldwide. It's primarily used for culinary purposes, providing flavour and garnish. Every part of the plant, including stems, leaves, and taproots, can be used to enhance the taste of various dishes (Marthe, 2020) .Parsley has been globally cultivated and used for millennia as a food flavouring. It is a globally popular spice that finds its way into various dishes. It is commonly used in salads, soups, sauces, and omelettes to add flavour and a fresh, aromatic touch. It's also frequently used to make herb butter and is a versatile accompaniment to a wide range of other dishes, enhancing their taste and presentation (Sarwar et al., 2016; Živković et al., 2021). Parsley has a long history of cultivation and uses across the world, primarily for enhancing the flavour of food. Its culinary applications have been appreciated for thousands of years. The scientific name for parsley, "petroselinum," has an interesting etymology. It is derived from the Greek words: "petra," which means "stone" or "rock," and "selinin," which means "celery." (Sarwar et al., 2016). Gathering wild parsley requires caution due to its resemblance to three toxic plants. *Aethusa cynapium*, known as fool's parsley, dog poison, or small hemlock (a poisonous plant used as a strong sedative), can be differentiated by white flowers and its shiny, yellow-green leaf undersides and unlike parsley's yellowish flowers with dull leaves. The second toxic plant is *Conium maculatum* (poison hemlock or poison parsley), and the third is *Cicuta maculata* (water hemlock). These cause some symptoms like weakness, vomiting, diarrhoea, paralysis, dilated pupils, and, in severe cases, death. (*Parsley Uses, Benefits & Side Effects - Drugs.Com Herbal Database*, 2023)

1.3.2 Botanical characteristics of beetroot

Beta vulgaris, commonly known as beetroot, belongs to the Chenopodiaceae family and is widely used as a natural food colourant. Its distinctive colour comes from betalains, which are nitrogen-containing, water-soluble pigments and they are responsible for the vibrant red, purple, and yellow colours seen in many fruits, vegetables, and flowers (Punia Bangar et al., 2023). *Beta vulgaris*, like many others in the family of Chenopodiaceae, is a halophyte, and it is a very variable species with four main groups of agricultural significance, including fodder beets, garden beets and sugar beet. Sugar beet is a biennial plant that, in Egyptian crop rotation, has a crucial position as a winter crop because of its ability to grow in poor, calcareous and saline-alkaline soils (El-Emary, 2017). Also, this family is distributed across diverse environments, particularly in steppe, desert, and saline-alkaline regions. They are commonly found in southern America, Central Asia, South Africa, and Oceania, as well as along the coasts of the Mediterranean, Caspian, and Red Seas. These plants are well-adapted to harsh conditions, including saline soils, making them prevalent in such challenging ecosystems (Abd El-Wahab et al., 2022).

1.3.3 Botanical characteristics of broccoli

The origin and use of the name "broccoli" are explored, along with a proposed distinction between cauliflower and broccoli based on their developmental differences at marketable maturity (Gray, 1982) . Broccoli develops green flower heads consisting of tightly packed clusters that grow on several branching stems (Gray, 1982) . Broccoli is an Italian word from the Latin brachium, meaning an arm or branch (Nagraj et al., 2020) . In Italy, "broccoli" refers to the young, edible flower shoots of *Brassica* plants, such as cabbages and turnips. While it originally described sprouting varieties, the term now also includes heading types, which produce a large, singular terminal inflorescence (Gray, 1982.). In var. *italica*, the characteristic colour variations of the head arise from pigments located in the sepals of the flower buds; these pigments not only contribute to the visual appeal of the broccoli but also play a role in its overall health and nutritional profile, as they can possess antioxidant properties (Gray, 1982).

1.3.4 Botanical characteristics of cauliflower

Brassica family, which encompass various types of cauliflower, broccoli, cabbage, kale and Brussels sprouts, are eaten globally (Podsedek, 2007). Cauliflower is a winter vegetable crop that belongs to the Brassicaceae family. It is cultivated in various countries, including several European nations, the United States and Asia (Singh et al., 2013). The cauliflower curd consists of a cluster of short, thick peduncles that bear pre-floral meristems as the area of cellular division. Although curds can appear in a variety of colours, the Mediterranean region is known for a small number of distinct types featuring vibrant green, purple, or yellow hues. In contrast, most other major cauliflower-growing regions primarily accept only white or very pale cream curds as commercially viable (Crisp et al., 1975).

1.3.5 Botanical characteristics of carrot

The carrot (*Daucus carota L.)* belongs to the family of Apiaceae (BAO et al.,1994) and is native to Asia, Europe, Africa, and Macaronesia (Glišić et al., 2007). The wild carrot, often referred to as Bird's Nest, Bishop's Lace is typically a biennial plant that grows to about 1 meter in height. It features unique clusters of small white flowers that bloom in warmer months. In contrast to cultivated carrots, wild carrots possess a slender taproot, hairy stems, and leaves that are two- to three-pinnate, along with pinnate bracts situated beneath a concave umbel when in fruit. Additionally, the fruits are spiny. This plant flowers from May through September (Ismail et al., 2023). Carrots are widely cultivated for their edible taproots, which vary in size and shape across different varieties. These roots can range from round and globular to elongated and slender, with ends that may be blunt or tapered to a point. While the most common colour is orange, other cultivars produce roots in shades of purple, yellow, and white. Optimal growing conditions for carrots include cool to moderate temperatures, as they are sensitive to heat and do not grow in hot climates. The plants also need deep, nutrient-rich, loose soil that allows for easy root development. For optimal freshness, carrots should feel firm, with smooth, blemish-free skin. A vivid orange colour indicates a high level of beta-carotene, a valuable nutrient converted into vitamin A by the body. Smaller varieties of carrots are typically the most tender (Carrot | Description, Domestication, & Cultivation | Britannica, n.d.).

1.4 Nutritional values of vegetables used in this work

1.4.1 Nutritional values and mineral content of parsley

Petroselinum crispum stands as a remarkable reservoir of antioxidants, offering a spectrum of advantageous attributes. Among its merits, there are antioxidative, pain-relieving, muscle-relaxing, blood sugar-regulating, immune system-enhancing, and digestive benefits.(Es-Safi et al., 2021; Farzaei et al., 2013) Moreover, this herb boasts a wealth of antioxidant nutrients, including β-carotene, vitamin A, vitamin E, vitamin K, vitamin C, lutein, cryptoxanthin, zeaxanthin and folates. Parsley abounds in polyphenolic flavonoid antioxidants like crisoeriol, apigenin, luteolin, and apiin (Ajmera et al., 2019). Tables 1 and 2 report them in detail. In addition, table 1 shows some bioactive elements extracted from *Petroselinum crispum* and their therapeutic significance.(Malik et al., 2014)

Parsley plants contain various bioactive compounds in different parts, including the leaves, stems, and roots. As well-reviewed by (Agyare et al., 2017; Dadan et al., 2018; Fernandes et al., 2020) , these compounds include:

- **Furanocoumarins**: These compounds, comprising xanthoxin, trioxalen, and angelicin, are known for their potential medicinal properties. They can have anti-inflammatory and antimicrobial effects and may also interact with certain enzymes in the body.
- **Essential Oils**: Parsley contains a variety of essential oils with diverse chemical components, such as hydrocarbons, alcohols, aldehydes, and aromatic compounds. These oils can contribute to the plant's flavour and fragrance. Some of them may have antimicrobial and antioxidant properties.
- **Flavonoids**: Flavonoids like quercetin, apigenin, and luteolin, as well as their glycosides, are powerful antioxidants. They may help protect cells from damage, reduce inflammation, and have potential benefits for cardiovascular health.

- **Carotenoids**: Carotenoids, such as neoxanthin, β-carotene, lutein, and violaxanthin, are responsible for the colouration of parsley. They are precursors to vitamin A and are known for their antioxidant properties, which can be beneficial for eye health and immune function.
- **Vitamins**: Parsley is a good source of vitamins, including vitamins A, C, and various B-complex vitamins. These vitamins play crucial roles in immune support, skin health, and overall well-being.
- **Minerals**: The plant also provides essential minerals like iron, zinc, calcium, and phosphorus. Iron is important for oxygen transport in the body, while calcium and phosphorus are essential for bone health. Zinc plays a role in immune function and wound healing.
- **Fatty Acids**: The presence of fatty acids like linolenic and palmitic acid indicates the potential for some healthy fats in parsley, which can be beneficial for various cellular functions.

Parsley oil can lead to adverse effects such as headache, giddiness, loss of balance, convulsions, and renal damage. Psoralen compounds in parsley have been associated with photodermatitis reactions in parsley cutters. When used as a food ingredient, parsley is generally considered safe. However, using it in dosages higher than those found in foods is unproven in terms of safety and efficacy and should be avoided. In higher doses, parsley can act as an emmenagogue (stimulating menstrual flow) and even have abortive effects. While no major toxicities are typically reported with parsley use, pregnant women should avoid it due to potential uterotonic (uterine-stimulating) effects(*Parsley Uses, Benefits & Side Effects - Drugs.Com Herbal Database*, 2023)

Table 2 reports the amounts of various components in both fresh and freeze-dried parsley (Food Data Center of America) (*U.S. Department of Agriculture*, n.d.). This information clarifies the nutritional content and composition differences between the two forms of parsley.

1.4.2 Nutritional values and mineral content of beetroot

Beetroot is packed with minerals and contains significant amounts of phenolic compounds and antioxidants such as carotenoids, coumarins, sesquiterpenoids, triterpenes, and flavonoids like tiliroside, astragalin, kaempferol, rhamnocitrin and rhamnetin (Punia Bangar et al., 2023).Table 3 shows some Phenolic compounds of Beetroot. Also, it is a rich source of essential nutrients, offering carbohydrates, proteins, dietary fibre, sucrose and a variety of vitamins, including vitamin C and B-complex. The wide range of phytochemicals in beetroot makes it a valuable contributor to nutraceuticals, enabling the production of functional foods with health-enhancing benefits (Punia Bangar et al., 2023) In addition to these, sugar beet cultivars are exhibiting for having the highest concentrations of potassium and nitrogen and the lowest levels of phosphorus in their leaves (Abd El-Wahab et al., 2022) .Table 4 shows the nutrients of Beetroot according to USDA Food Data Central (*Beets, Usda.Gov*, n.d.)

1.4.3 Nutritional values and mineral content of broccoli

Raw *Brassica* vegetables contain high fibre, low calorie, and essential antioxidants like isothiocyanates and phytochemicals (Podsedek et al., 2007).

Vitamin C and Phenolic compounds are the primary antioxidants in *Brassica* vegetables, attributed to their high concentrations and strong antioxidant properties. In contrast, lipid-soluble antioxidants such as carotenoids and vitamin E account for up to 20% of the total antioxidant activity in this family (Podsedek et al., 2007).

The polyphenol content in vegetables, similar to other phytochemicals, can be affected by several factors, including harvest maturity, plant varieties, climate conditions, storage ways and farming practices (Podsedek et al., 2007). Also, some research indicated that salinity stress can influence the phenolic compound levels in plants, though the effect largely depends on the species' sensitivity to salt. As a result, when aiming to produce broccoli with enhanced functional ingredients or higher

phytochemical content, it is essential to consider agronomic factors and the degree of saline stress applied (López-Berenguer et al., 2009). This stress can lead to variations in bioactive compounds, with the effects differing significantly across different plant organs. Although salinity often reduces growth, it has also been shown to enhance the nutritional quality of edible inflorescences (López-Berenguer et al., 2009). Table 5 shows the nutrients of Broccoli according to USDA Food Data Central.

1.4.4 Nutritional values and mineral content of cauliflower

Cauliflower (*Brassica oleracea* var. *botrytis*), belonging to the Brassicaceae family, is a highly popular vegetable enjoyed in various global cuisines. Nutritionally, it consists of up to 91% water, 4.5% sugar, 2.5% protein, 1.8% crude fibre, and just 0.3% fat. It is a good source of essential minerals such as phosphorus, potassium, sodium, calcium, sulfur, iron, and magnesium. In addition to these nutrients, cauliflower is packed with beneficial phytochemicals and vitamins, including vitamin C, vitamin B12, and PP, among others (Uher et al., 2017). A single cup of boiled cauliflower provides an excellent source of folate, vitamin C, and dietary fibre (Sultana et al., 2019). Ahmed et al.2017 reported that raw cauliflower contains 782.43 mg of total polyphenols per 100 g (dry mass basis). Still, in another study, the fresh cauliflower exhibited a slightly higher concentration of 886.4 mg. It's essential to note that the levels of these compounds are largely influenced by agricultural practices, environmental factors during cultivation, and the specific cultivar of the vegetable (Kapusta-Duch et al., 2017).

Table 6 shows the nutrients of cauliflower according to USDA Food Data Central

1.4.5 Nutritional values and mineral content of carrot

Daucus carota, commonly known as carrot, is an Apiaceae species native to Europe, Asia, Africa, and Macaronesia. In traditional medicine, extracts of *Daucus carota* are utilised for treating hepatic and renal insufficiencies, as well as various skin disorders, including burns and furunculosis (Glišić et al., 2007). Carrot is an edible root vegetable and a rich source of vitamins A, B, and C, anthocyanins, as well as carotenoids (α, β, and ƛ carotene) (BAO & CHANG, 1994). A vivid orange colour indicates a high level of beta-carotene, a valuable nutrient converted into vitamin A by the body (*Carrot | Description, Domestication, & Cultivation | Britannica*, 2024). Apiaceae family are commonly recognized for their antioxidant and anti-inflammatory properties, and they have been utilized in the treatment of infections, and respiratory conditions, and are known to exhibit hepatoprotective effects (Ayeni et al., 2018; Kataria et al., 2016). Traditionally, all parts of the carrot have been used for their medicinal properties, serving as diuretics, aphrodisiacs, antidiabetics, and remedies for muscle pain. Additionally, a boiled extract of carrot leaves has been consumed orally as a uterine stimulant during childbirth and is known to have abortifacient potential (Ayeni et al., 2018; Ross, 2005). Besides this, certain species of carrots possess antimicrobial properties and have been traditionally used to flavour alcoholic beverages (Ross, 2005). Essential oil derived from carrot seeds is widely utilized as a flavouring agent across various food categories and as a fragrance component in cosmetics, soaps, and perfumes, also, this oil is claimed to have several biological properties, including antimicrobial effects, hepatocellular regeneration, general toning and stimulating effects, cholesterol regulation, and cicatrisation (Maxia et al., 2009). The aerial parts of Carrot are nutrient-rich, so they can be used as livestock feed supplements as well as vegetables for human consumption. Incorporating these parts into daily diets could enhance food security in developing countries, particularly those facing significant poverty challenges (Ayeni et al., 2018). Table 7 shows the nutrients of Beetroot according to USDA Food Data Central.

1.5 Pharmacological activities of vegetables used in this work

1.5.1 Pharmacological activities of parsley

The medicinal herb parsley has a rich history of usage over 2000 years ago in the Mediterranean region, dating back to its initial use by the Ancient Greeks in religious ceremonies. When Hippocrates introduced Parsley as a diuretic it gained further recognition and application. (Živković et al., 2021)

This versatile herb offers a wide range of health benefits, making it highly regarded in traditional and modern medicine. Some of its key attributes are as follows: (Butu & Rodino, 2019)

- **Effectiveness in Rickets**: Parsley has demonstrated its effectiveness in addressing rickets, a condition associated with vitamin D and calcium deficiencies, which can lead to weak or brittle bones.
- **Role in oxygen metabolism**: It aids in efficiently utilising oxygen in the body.
- **Support for Adrenal and Thyroid Function**: Helping to regulate various physiological processes, including metabolism and stress response.
- **Vascular Health**: Parsley has a positive impact on blood vessels, especially capillaries.
- **Genitourinary Tract Health**: Parsley is particularly beneficial for the genitourinary system by preventing and alleviating issues related to this system, including kidney and gallbladder stones.
- **Eye Disorders**: Due to its rich content of vitamins and antioxidants it has shown effectiveness in preventing eye disorders.
- **Anti-Aging Effects**: Regular consumption of parsley can help in preventing premature aging because of its antioxidant properties that combat oxidative stress and free radical damage.
- **Antiseptic Properties**: Parsley juice is recognised as an excellent antiseptic for the blood and intestines by cleaning and detoxifying these vital systems.

- **Potential Cancer Prevention**: It is believed that its antioxidant and anti-inflammatory properties may play a role in reducing cancer risk. (Butu & Rodino, 2019)

  Table 1 reveals the therapeutic significance of specific components found in *Petroselinum crispum* and the potential health benefits associated with its various constituents.

1.5.2 Pharmacological activities of beetroot

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- **Vascular Health**: Parsley has a positive impact on blood vessels, especially capillaries.
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Table 1 reveals the therapeutic significance of specific components found in *Petroselinum crispum* and the potential health benefits associated with its various constituents.

1.5.3 Pharmacological activities of broccoli

A wide array of antimicrobial agents, sourced from traditional medicinal plants, is available for treating numerous diseases caused by microorganisms (Ravikumar, 2015). Although currently, at least 25% of all new-generation medicines are derived from medicinal plants(*Traditional Medicine in the WHO South-East Asia Region: Review of Progress 2014–2019*, n.d.), and only a limited number are utilised specifically for their antimicrobial effects. Plants are a rich source of diverse secondary metabolites, and many have demonstrated significant antimicrobial properties. These compounds can be categorised into various groups based on their structure and activity, including flavonoids, alkaloids, phenolics, polypeptides, polyphenols, essential oils, and tannins. Together, these phytochemicals form a powerful toolkit against microbial infections, representing an area of untapped potential in modern medicine (Perumal Samy & Gopalakrishnakone, 2010). Broccoli also offers numerous health benefits, mainly because of its antioxidant and anti-carcinogenic compounds. It primarily comprises sulforaphane, polyphenols, selenium and glucosinolates, all of which contribute to its protective effects against various diseases (Mahn & Reyes, 2012). Broccoli is recognised for its phytotherapeutic benefits, particularly in skin health. It is believed that Broccoli possesses properties that may aid in reducing the appearance of warts and promoting healthier skin. Additionally, the nutritional composition of broccoli enhances its overall effectiveness in supporting skin health, making it a valuable

addition to natural remedies (Moreno et al., 2006). Broccoli contains a high concentration of flavonoids, known for their anti-inflammatory and antioxidant effects, which help to guard against diabetes. Research suggests that these flavonoids may contribute to a lower risk of developing the disease (Nettleton et al., 2006). Sulforaphane, a compound found in broccoli, can activate various specific proteins called peroxisome proliferator-activated receptors (PPARs). These proteins help regulate blood sugar levels, especially when blood sugar is high or when there's oxidative stress (cell damage). Sulforaphane's effects are beneficial for people with diabetes because it can help prevent problems like blood vessel damage, tissue scarring (fibrosis), and kidney issues. Because of these benefits, sulforaphane is seen as an excellent supplement to help manage type 2 diabetes (Bahadoran et al., 2013). Broccoli sprout is an abundant source of various isothiocyanates (ITCs), recognised as a significant class of cancer preventive agents. These compounds have been shown to inhibit the size, multiplicity, and progression of bladder cancer when the extracts are selectively delivered to the bladder epithelium via urinary excretion (Bahadoran et al., 2013; Ravikumar, n.d.). Sulforaphane available in broccoli, in combination with other phytochemicals like indole-3-carbinol and brassinin found in broccoli, has shown promise in the realm of cancer chemoprevention. Given their safety profiles, cost-effectiveness, and oral bioavailability, these phytochemicals present significant potential for cancer prevention strategies (Gullett et al., 2010).

1.5.4 Pharmacological activities of cauliflower

Cauliflower is rich in sulforaphane, a compound with anticancer solid properties. It inhibits growth in various cancerous cell lines, including colon, lung, prostate, and breast cancers. Sulforaphane promotes cancer cell death, regulates key pathways, and reduces inflammation and oxidative stress, making it a valuable natural agent for cancer prevention (IIDA et al., 2021; Rutz et al., 2020). A recent study by Zhou et al (Zhou et al., 2022) highlighted the effectiveness of sulforaphane, a natural compound found in some vegetables like Cauliflowers, in treating breast cancer.

This compound not only inhibits the proliferation and activity of breast cancer cells but also specifically modulates the NF-κB/MMP-9 signalling pathway, a crucial pathway involved in cancer cell growth, migration, and invasion. So, sulforaphane disrupts the cellular mechanisms that allow cancer cells to spread, making it a potential therapeutic agent. Additionally, sulforaphane reduces inflammation and oxidative stress in the tumour microenvironment, further enhancing its anti-cancer properties and making it a promising candidate for complementary cancer therapy (Zhou et al., 2022).

1.5.5 Pharmacological activities of carrot

The aerial parts of *Daucus carota* L. subsp. *carota* (wild carrots) are nutrient-rich, making them suitable for livestock feed and human consumption (Ayeni et al., 2018). The roots of *Daucus carota* contain three new sesquiterpene daucane (a specific type of organic compound found in plants, particularly in the carrot family) derivatives along with four known compounds. These compounds demonstrated significant antifungal activity, particularly against *Fusarium oxysporum* and *Aspergillus niger*, indicating potential for therapeutic or agricultural applications (Maxia et al., 2009)

Also, Methanol extracts from *Daucus carota* seeds exhibit antibacterial activity against various bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Lactobacillus plantarum* and *Citrobacter freundii*, highlighting their potential for natural antimicrobial applications (Kumarasamy et al., 2005). The antifungal properties of various *Daucus carota* subspecies were studied against different fungal strains. Oil extracts from these plants displayed both fungistatic and fungicidal effects, with *Fulvia fulvum* being the most susceptible and *Trichoderma viride* and *Aspergillus ochraceus* the most resistant. Among the plant parts, oil from unripe fruits was the most effective, followed by oils from ripe fruits, roots, stems, leaves, and flowers (Ismail et al., 2023). Additionally, carrot has the antifungal

activity of essential oils from blooming and flowering umbels of *Daucus carota* ssp showed more potent antifungal effects against *Cryptococcus neoformans* and dermatophyte strains than against *Candida* and *Aspergillus* strains (Alves-Silva et al., 2016; Maxia et al., 2009)

Numerous studies have confirmed the anti-inflammatory properties of terpenes derived from carrots. Specifically, (+)-α-pinene has demonstrated effectiveness in inhibiting IL-1β-stimulated inflammatory and catabolic pathways, indicating its potential for anti-osteoarthritic effects. This terpene also reduces the production of pro-inflammatory cytokines such as IL-6 and TNF-α in macrophages from rats, alongside a decrease in nitrite production, which is associated with inflammation (Alves-Silva et al., 2016; Soković et al., 2009).

Also, geranyl acetate available in carrots has shown significant anti-inflammatory activity in vitro, effectively reducing inflammation without compromising the viability of macrophages and keratinocytes. These findings suggest that the terpenes in carrots may play a crucial role in mitigating inflammatory responses, supporting their potential use in therapeutic applications (Maxia et al., 2009)

Besides this, α-pinene and geranyl acetate were highlighted as the main components responsible for the antibacterial effectiveness, aligning with previous research that underscores the antibacterial properties of α-pinene (Ismail et al., 2023).

1.6 Effects of freezing and drying vegetables used in this work

1.6.1 Effects of freezing and drying on Parsley

In the context of plant-based foods, several factors come into play to ensure the retention of high-quality attributes when the product is thawed. These factors encompass not only the freezing process and the frozen state but also extend to considerations such as the initial quality of the raw materials, post-harvest handling, and any pre-treatments applied before freezing. All these elements collectively influence the overall quality of the frozen product while thawing. (Bonat Celli et al., 2016; Neri et al., 2020a)

For certain herbs like parsley and thyme, the choice of drying method significantly impacts their aromatic profiles. Air drying at room temperature emerged as the method of choice, preserving the aromatic constituents and overall aroma quality better than alternatives (Mangkoltriluk et al., 2005; Petropoulos et al., 2010). When subjected to higher drying temperatures e.g., 45°C, 55°C, or 65°C, these herbs experienced a reduction in certain components, but this didn't cause a noticeable difference in organoleptic properties. (Petropoulos et al., 2010; Venskutonis, 1997)

The transition of water to ice is a complex phenomenon characterized by two distinct stages: nucleation and crystal growth.(Kiani & Sun, 2011; Neri et al., 2020a) This phenomenon is partly attributed to the unique behaviour of water in plant tissues. The cellular organization leads to the crystallization of local freezable water within and outside the cells. Due to osmotic pressure differentials, unfrozen water migrates towards the frozen regions within the cells and their organelles. In plant-based foods, water moves from the vacuole, passing through the tonoplast, into the cytoplasm, and then into the intercellular spaces by traversing cell membranes and cell walls (Li et al., 2018; Neri et al., 2020).

In such conditions, liquid water is drawn out from supercooled cells, resulting in tissue dehydration—a phenomenon known as freeze concentration. Additionally, this process leads to cell separation in the middle lamella region, culminating in cell wall rupture, cell shrinkage, and eventual cell wall collapse (Neri et al., 2020; Yamada et al., 2002).

In order to survey the influence of freezing on Parsley, an analysis of the essential oil compositions in the leaves of two different parsley cultivars was conducted at two specific stages: one after two weeks of being stored in a freezer and the other after four weeks of air drying (Masanetz & Grosch, 1998; Petropoulos et al., 2010). These findings were then compared to the composition of essential oils in fresh parsley leaves. The results revealed distinct changes in the composition of these oils depending on the processing method and the parsley variety. In the case of plain-leafed parsley, freezing led to a marginal reduction in the concentrations of both β-phellandrene and 1,3,8-p-menthatriene compared to fresh leaves. However, the drying process had contrasting effects on these components. It caused an increase in the levels of β-phellandrene but resulted in a substantial decrease in 1,3,8-p-menthatriene. Conversely, when analysing turnip-rooted parsley leaves, the outcomes were different. Freezing caused a significant decline in the concentration of 1,3,8-p-menthatriene, while air drying led to an increase in its levels. β-phellandrene showed a slight reduction during both processes, whether freezing or drying. Additionally, two other components, p-α-dimethylstyrene and terpinolene, experienced decreases only when the leaves were subjected to the drying process. (Masanetz & Grosch, 1998; Petropoulos et al., 2010) The effects of prolonged freezing and air drying on the composition of essential oils in both plain-leafed and turnip-rooted parsley leaves are observed. The findings shed light on how these storage conditions and durations impact specific chemical components within the essential oils. Petropoulos et al. (2010) (Petropoulos et al., 2010) observed that parsley leaf storage impacts essential oil composition. In frozen plain-leafed parsley (-10°C for two months), 1,3,8-p-menthatriene levels dropped, while apiole and myristicin increased, with a stability of β-phellandrene remaining. For frozen turnip-rooted parsley, β-myrcene and β-phellandrene rose slightly. In air-dried plain-leafed parsley (stored at 24°C), β-phellandrene decreased after one month, and by two months, 1,3,8-p-menthatriene dropped while β-phellandrene and β-myrcene increased. Meanwhile, air-dried turnip-rooted parsley showed a steady decline in 1,3,8-p-menthatriene and increases in β-myrcene and β-phellandrene over time. These findings illustrate how storage conditions influence parsley's essential oil profile (Petropoulos et al., 2010).

1.6.2 Effects of freezing and drying on beetroot

Freeze-drying is the best dehydration method for heat-sensitive raw materials, especially pigments of beetroot. This procedure produces good-quality food with well-retained flavour and nutritional quality and maintains the material volume with porous structures and good rehydration ability (Janiszewska-Turak et al., 2021).

During the drying process, cell shrinkage, the reduction in cell size that occurs by losing water from a material, is a critical phenomenon. It causes substantial changes to the structure of the material, allowing water to escape more easily (Ramos et al., 2004).

Pigment use in food production is growing, with a preference for natural sources over synthetics due to potential health risks associated with artificial options. Ingredients like beetroot provide natural pigments, which generally show minimal change after freeze-drying. Although slight pigment variations were observed, these shifts were mainly statistically insignificant, aside from a marked change in betalain (red) pigment levels in the pomace of beetroot (Janiszewska-Turak et al., 2021).

A research study by Dalmau et al (Dalmau et al., 2019a) revealed that freezing changed the microstructure and composition of frozen beetroots compared to raw beetroots. Structural analysis showed that freezing and simulated gastric digestion caused significant cell damage, altering how beetroots behave during digestion. Frozen beetroots generally absorbed more moisture and became more acidic than raw beetroots during digestion. While freezing reduced total polyphenol content and antioxidant activity in beetroots initially, it also improved the release and bioavailability of these compounds during digestion. The increase in polyphenols and antioxidants in gastric juice was greater than the reduction within the beetroot itself. Notably, freezing with liquid nitrogen led to a larger loss of phenolic compounds in the beetroot but a greater release of these into gastric juice (Dalmau et al., 2019).

1.6.3 Effects of freezing and drying on broccoli

Freezing is widely used for long-term vegetable preservation. For broccoli, blanching is performed before freezing to deactivate enzymes that could lead to quality loss and to help maintain colour, flavour, and nutritional value. Blanching also removes trapped air and reduces microbial load, improving the stability of the frozen product. Industrial blanching typically involves heating broccoli at high temperatures (85–100°C) using either hot water or steam. This step is essential for minimizing enzyme activity and enhancing the preservation of sensory and nutritional attributes during storage (Bahçeci et al., 2005). In a study by González-Hidalgo et al (González-Hidalgo et al., 2019), the effects of industrial blanching and freezing on broccoli's physical and nutritional properties were evaluated. Key quality markers such as colour, texture, vitamin C forms (ascorbic and dehydroascorbic acids), glucosinolates, phenolic compounds, and antioxidant capacity were analyzed after processing. The findings indicated reductions in chroma (29%), firmness (50%), phenolics (57%), and vitamin C (30%). Despite these declines, antioxidant levels remained similar to fresh broccoli, thanks to contributions from various forms of vitamin C, specific phenolics, and flavonoids. Additionally, glucosinolate levels were unaffected, suggesting that industrial processing effectively preserves broccoli's antioxidants and phytochemicals, making the frozen product nutritionally comparable to its fresh counterpart (González-Hidalgo et al., 2019). Colour and texture are critical quality attributes influencing consumer acceptance of vegetables. Textural degradation during processing stages and such as blanching, freezing, and thawing can result from multiple factors. These include dehydration, drip loss, and structural damage to cell tissues. Particularly, the formation of ice crystals during freezing often leads to mechanical damage, which compromises the cellular integrity of the vegetable tissue. Such textural changes reduce overall quality and can impact the sensory experience, underscoring the importance of optimizing processing conditions to preserve these attributes (Kidmose, U., & Martens, H. J. 1999.). For high-quality products, freeze-drying is ideal because it uses low temperatures and pressure to remove water through sublimation, preserving the food's structure, nutrients, and flavour (Liu et al., 2017) . In dried foods, two key factors are essential for maintaining

safety and quality: the amount of leftover moisture and the water activity level. For dried fruits and vegetables, it's important to keep moisture levels low. Additionally, the water activity should not exceed 0.6. Keeping water activity at or below this level helps prevent the growth of harmful bacteria, moulds, and yeast, which ensures the product stays safe and stable during storage (Ozcelik et al., 2020)

The cooking methods by Pellegrini et al.(Pellegrini et al., 2010) reported that boiling and oven-steaming caused increases in the total phenolic content and antioxidant capacity of fresh broccoli while microwaving did not. On the other hand, Zhang and Hamauzu noted that both microwaving and boiling resulted in a decrease in the total phenolic content, total carotenoids, ascorbic acid levels, and antioxidant activity of fresh broccoli (D. Zhang & Hamauzu, 2004).

1.6.4 Effects of freezing and drying on cauliflower

The conflicting data concerning cauliflower primarily revolve around specific cooking methods, including boiling, steaming, and microwaving. Girgin and El (Girgin & El, 2015) demonstrated that steaming enhanced total phenolic content and antioxidant activity, whereas boiling led to decreased levels (Çubukçu et al., 2019a; Girgin & El, 2015), but Volden et al. observed that boiling, blanching, and steaming negatively impacted the total phenolic content, ascorbic acid levels, and antioxidant activity (Volden et al., 2009). Conversely, Dos Reis et al.(Dos Reis et al., 2015) indicated that while microwaving did not affect the phenolic content of cauliflower, boiling and steaming resulted in reductions of phenolic compounds; however, antioxidant activity increased only with microwaving (dos Reis et al., 2015).

Freezing cauliflower reduces its antioxidant activity and increases malondialdehyde levels (a marker of oxidative stress), although the total phenolic content remains mostly unchanged. This suggests that the loss in antioxidant activity may be due to decreases in non-phenolic compounds, as phenolic levels themselves don't significantly drop (Çubukçu et al., 2019).

After three months of frozen storage, cauliflower samples possessed an average vitamin C content of only 11.9 mg per 100 g. In another study, a significant drop in vitamin C levels in broccoli was reported, with the final content being only 60% of the initial content (Kapusta-Duch et al., 2017).

1.6.5 Effects of freezing and drying on carrot

Carrots have a high content of water (about 86–93%), and therefore are prone to quality deterioration during transport and storage. Effective preservation methods include refrigeration to slow microbial growth, and Modified Atmosphere Packaging to reduce respiration rates. High relative humidity (90–95%) and edible coatings can minimise moisture loss, this strategy maintains carrot quality by preventing dehydration, microbial spoilage, and nutrient loss (Li et al., 2024).

Freeze drying is particularly effective for delicate products with sensitive surfaces, as it minimises structural damage and quality loss, resulting in a dried product that closely resembles its fresh counterpart in appearance, taste, and nutritional content (Qing-guo et al., 2006).Freeze drying stands out as an ideal preservation method for carrots to remove water through sublimation at low temperatures, preserving texture, colour, and nutrients far better than conventional drying methods, which often cause shrinkage or nutrient degradation (Qing-guo et al., 2006)

Image analysis combined with machine learning achieved 100% accuracy in distinguishing freeze-dried from fresh carrot slices, with top-performing algorithms like Random Forest and Logistic. Methods such as Genetic Search, Ranker, and Best First confirmed the procedure's effectiveness in assessing freeze-drying effects on carrot texture. This non-destructive approach has the potential for broader application in evaluating carrot processing stages and could be enhanced through deep learning and predictive models linking texture changes to chemical properties over time (Ropelewska et al., 2023).

A study by Araújo-Rodrigues et al. (2021) confirms that baby carrot by-products are valuable ingredients rich in bioactive compounds (phenolics, carotenoids, tocopherols) whether processed as pulps or powders. Both forms retain nutrients like fresh carrots. Freezing enhances vitamin E (tocopherol) levels in both vegetables but gradually reduces total polyphenolic content (TPC) and antioxidant capacity over time. While drying stabilizes antioxidant properties during storage, it significantly reduces carotenoids and polyphenols immediately after processing. Overall, pulps maintain a superior nutritional profile compared to powders, yet both forms offer functional, stable, and safe nutritional options, with promising economic and environmental benefits for by-product utilization (Araújo-Rodrigues et al., 2021).

Industrial potential of ozone pre-treatment for drying carrot cubes, showing that it enhances mass transfer rates, shortens drying times, and improves energy efficiency. The Lewis model best described the drying kinetics, with ozone pre-treatment (40 μg/L) leading to superior quality in dried carrot cubes by preserving phenolic content, β-carotene, and antioxidant activity. Additionally, it boosted the inhibitory activity of α-amylase and α-glucosidase and reduced gelatinization enthalpy. Overall, ozone pre-treatment is a promising, non-thermal method to improve both efficiency and quality in carrot drying processes (Santos et al., 2024).

1.7 Preparation of the plants for storage in the freezer

To prepare the plants for storage in the freezer, several steps are required, including the following: (Grzeszczuk et al., 2007; Lisiewska & Kmiecik, 1997)

- **Washing**: The initial step involves cleaning the fresh plant material to remove dirt, debris, and any surface contaminants. This step is essential for food safety and ensuring the produce is free from unwanted particles.
- **Blanching**: It involves briefly immersing the plant material in hot water at 95-97°C (203-207°F). The purpose of blanching is to inactivate enzymes in the plant material that can lead to quality loss over time. Enzyme inactivation helps preserve the colour, texture, and flavour of the plants.
- **Water-to-Plant Proportions**: The 2:1 proportion of water to plants means that for every unit of plant material, two units of water are used during blanching. This ratio is important to ensure effective blanching and enzyme inactivation.
- **Cooling**: After blanching, the plant material is quickly cooled in running water for about 2 minutes. This step halts the cooking process and helps to maintain the desired texture and colour.
- **Draining**: 30 minutes of draining is essential to remove excess water, which can lead to the formation of ice crystals during freezing. Excess water can also affect the texture of the frozen product.
- **Packaging**: The blanched, cooled, and drained plant material is then packed.
- **Freezing**: The packed plant material is stored in a freezing chamber at -25°C to -27°C (-13°F to -16.6°F) for a period of 3, 6, and 9 months. Freezing at these low temperatures helps to maintain the freshness and nutritional content of the plant material for an extended period.

Table 1: Bioactive components of *Petroselinum crispum* and their therapeutic significance.

Medicine applications	**Chemical component**	**References**
Diuretics to achieve blood pressure control	Myristicin and apiol	(Kreydiyyeh & Usta, 2002; Malik et al, 2014)
Prevent cancer in the prostate, lung, colon, breast, skin, brain, and tongue, Leukaemia, and initial stages of atherosclerosis.	Flavone apigenin	(Engelmann et al., 2002; Manthey & Guthrie, 2002; Walle & Walle, 2007);
Antioxidant properties	Flavonoids (apiin, luteolin, apigenin-glycosides), ascorbic acid, tocopherol and essential oils (apiole, myristicin)	(Ajmera et al., 2019; Fejes et al., 2000; Nielsen et al., 1999)
Antioxidant and antibacterial activities	Phenolic Compounds	(Wong & Kitts, 2006)
Used in dentistry as a local anaesthetic and antiseptic for oral health. reducing blood sugar levels in diabetics	Crucial oil like: Eugenol, Apiol, Limonene	(Ajmera et al., 2019)
Strong antiplatelet aggregation activity	Apigenin and cosmosiin	(Chaves et al., 2011; Malik et al, 2014)
Treatment of hyperuricemia	Kaempferol and Quercetin	(Haidari et al., 2011)
Suppress the immune response, allergy, autoimmune and chronic inflammatory disorders, Diuretic effect	Parsley essential oil	(Malik et al, 2014; Yousofi et al., 2012)

Table 2. Chemical components of both fresh and freeze-dried parsley (*U.S. Department of Agriculture*, n.d.)

Parsley, freeze-dried			**Parsley, fresh**		
Name	**Amount**	**Unit**	**Name**	**Amount**	**Unit**
Water	2	g	Water	87.7	g
Energy	271	kcal	Energy	36	kcal
Energy	1130	kJ	Energy	151	kJ
Protein	31.3	g	Protein	2.97	g
Total lipid (fat)	5.2	g	Total lipid (fat)	0.79	g
Ash	19.1	g	Ash	2.2	g
Carbohydrate	42.4	g	Carbohydrate	6.33	g
Fiber, total dietary	32.7	g	Fiber, total dietary	3.3	g
Calcium, Ca	176	mg	Calcium, Ca	138	mg
Iron, Fe	53.9	mg	Iron, Fe	6.2	mg
Magnesium, Mg	372	mg	Magnesium, Mg	50	mg
Phosphorus, P	548	mg	Phosphorus, P	58	mg
Potassium, K	6300	mg	Potassium, K	554	mg
Sodium, Na	391	mg	Sodium, Na	56	mg
Zinc, Zn	6.11	mg	Zinc, Zn	1.07	mg
Copper, Cu	0.459	mg	Copper, Cu	0.149	mg
Manganese, Mn	1.34	mg	Manganese, Mn	0.16	mg
Selenium, Se	32.3	µg	Selenium, Se	0.1	µg
Vitamin C, total	149	mg	Vitamin C, total	133	mg
Thiamin	1.04	mg	Thiamin	0.086	mg
Riboflavin	2.26	mg	Riboflavin	0.098	mg
Niacin	10.4	mg	Niacin	1.31	mg
Pantothenic acid	2.52	mg	Pantothenic acid	0.4	mg
Vitamin B-6	1.38	mg	Vitamin B-6	0.09	mg
Folate, total	194	µg	Folate, total	152	µg
Folic acid	0	µg	Folic acid	0	µg
Folate, food	194	µg	Folate, food	152	µg
Folate, DFE	194	µg	Folate, DFE	152	µg
Vitamin B-12	0	µg	Vitamin B-12	0	µg

Vitamin A, IU	63200	IU	Vitamin A, RAE	421	µg
Vitamin D	0	µg	Retinol	0	µg
Fatty acids, trans	0	g	Carotene, beta	5050	µg
Cholesterol	0	mg	Carotene, alpha	0	µg
Tryptophan	0.516	g	Cryptoxanthin, beta	0	µg
Lysine	3.12	g	Vitamin A, IU	8420	IU
Methionine	0.21	g	Lycopene	0	µg
			Lutein + zeaxanthin	5560	µg
			Vit E (α-tocopherol)	0.75	mg
			Tocopherol, gamma	0.53	mg
			VitaminD (D2+D3)	0	µg
			Vitamin K (phylloquinone)	1640	µg
			Fatty acids, total trans	0	g
			Cholesterol	0	mg
			Tryptophan	0.045	g
			Lysine	0.181	g
			Methionine	0.042	g
			Threonine	0.122	g
			Isoleucine	0.118	g
			Leucine	0.204	g
			Cystine	0.014	g
			Phenylalanine	0.145	g
			Tyrosine	0.082	g
			Valine	0.172	g
			Arginine	0.122	g
			Histidine	0.061	g
			Alanine	0.195	g
			Aspartic acid	0.294	g
			Glutamic acid	0.249	g
			Glycine	0.145	g
			Proline	0.213	g
			Serine	0.136	g

Table 3: Phenolic compound of Beetroot

Phenolic compounds	**References**
Flavonoids: Betagarin Betavulgarin cochliophilin A dihydroisorhamnetinas 2,5-dihydroxy 6 7-methylenedioxyisoflavone, 3,5 dihydroxy-6,7-methylenedioxyflavanone 5-hydroxy-6,7-methylenedioxyflavone rutin quercetin 4′hydroxy-5-methoxy-6,7-methylenedioxy flavanone	(Hadipour et al., 2020; Vulić et al., 2014)
phenolic derivatives: 5,50,6,60-tetrahydroxy-3,3′biindolyl 5,6-dihydroxyindolecarboxylic acid N-trans-feruloyltyramine N-trans-feruloylhomovanillylamine Epicatechin, catechin hydrate vanillic acid, p-coumaric acid protocatechuic, caffeic acid syringic acid, proline, dehydro vomifoliol 4-hydroxybenzoic acid chlorogenic acid, ferulic acid	(Georgiev et al., 2010; Hadipour et al., 2020; Nemzer et al., 2011; Tamilselvan & Kumar, 2014)
Saponins, Betavulgarosides (I to X), oleanolic acid, hederagenin, akebonoic acid , gypsogenin	(Hadipour et al., 2020; Mikołajczyk-Bator et al., 2016; Mroczek et al., 2012)

Table 4. Chemical components of beetroot (Beets, Usda.Gov)

Name	Amount	Unit		Name	Amount	Unit
Water	87.6	g		Vitamin A, IU	33	IU
Energy	43	kcal		Lycopene	0	µg
Energy	180	kJ		Lutein + zeaxanthin	0	µg
Protein	1.61	g		Vitamin E (α-tocopherol)	0.04	mg
Total lipid (fat)	0.17	g		Vitamin E, added	0	mg
Ash	1.08	g		Vitamin D (D2 + D3)	0	IU
Carbohydrate	9.56	g		Vitamin D (D2 + D3)	0	µg
Fiber, total dietary	2.8	g		Vitamin K (phylloquinone)	0.2	µg
Total Sugars	6.76	g		Fatty acids, total saturated	0.027	g
Calcium, Ca	16	mg		Tryptophan	0.019	g
Iron, Fe	0.8	mg		Threonine	0.047	g
Magnesium, Mg	23	mg		Isoleucine	0.048	g
Phosphorus, P	40	mg		Leucine	0.068	g
Potassium, K	325	mg		Lysine	0.058	g
Sodium, Na	78	mg		Methionine	0.018	g
Zinc, Zn	0.35	mg		Cystine	0.019	g
Copper, Cu	0.075	mg		Phenylalanine	0.046	g
Manganese, Mn	0.329	mg		Tyrosine	0.038	g
Selenium, Se	0.7	µg		Valine	0.056	g
Vitamin C, total	4.9	mg		Arginine	0.042	g
Thiamin	0.031	mg		Histidine	0.021	g
Riboflavin	0.04	mg		Alanine	0.06	g
Niacin	0.334	mg		Aspartic acid	0.116	g
Pantothenic acid	0.155	mg		Glutamic acid	0.428	g
Vitamin B-6	0.067	mg		Glycine	0.031	g
Folate, total	109	µg		Proline	0.042	g
Folic acid	0	µg		Serine	0.059	g
Folate, food	109	µg		Fatty acids, total trans	0	g
Folate, DFE	109	µg		Cholesterol	0	mg
Choline, total	6	mg		Vitamin A, RAE	2	µg
Betaine	129	mg		Retinol	0	µg
Vitamin B-12	0	µg		Carotene, beta	20	µg

Table 5. Chemical composition of broccoli (*Broccoli, Usda.Gov*, n.d.)

Name	Amount	Unit		Name	Amount	Unit
Water	89.3	g		Choline, total	18.7	mg
Energy	34	kcal		Betaine	0.1	mg
Energy	141	kJ		Vitamin B-12	0	µg
Protein	2.82	g		Vitamin B-12, added	0	µg
Total lipid (fat)	0.37	g		Vitamin A, RAE	31	µg
Ash	0.87	g		Retinol	0	µg
Carbohydrate	6.64	g		Carotene, beta	361	µg
Fiber, total dietary	2.6	g		Carotene, alpha	25	µg
Total Sugars	1.7	g		Cryptoxanthin, beta	1	µg
Sucrose	0.1	g		Vitamin A, IU	623	IU
Glucose	0.49	g		Lycopene	0	µg
Fructose	0.68	g		Lutein + zeaxanthin	1400	µg
Lactose	0.21	g		Vitamin E (α-tocopherol)	0.78	mg
Maltose	0.21	g		Vitamin E, added	0	mg
Galactose	0	g		Tocopherol, beta	0.01	mg
Starch	0	g		Tocopherol, gamma	0.17	mg
Calcium, Ca	47	mg		Tocopherol, delta	0	mg
Iron, Fe	0.73	mg		Tocotrienol, alpha	0.04	mg
Magnesium, Mg	21	mg		Tocotrienol, beta	0	mg
Phosphorus, P	66	mg		Tocotrienol, gamma	0	mg
Potassium, K	316	mg		Tocotrienol, delta	0	mg
Sodium, Na	33	mg		Vitamin D (D2 + D3)	0	IU
Zinc, Zn	0.41	mg		Vitamin K (phylloquinone)	102	µg
Copper, Cu	0.049	mg		Vitamin K (Dihydrophylloquinone)	0	µg
Manganese, Mn	0.21	mg		Fatty acids, total saturated	0.114	g

Selenium, Se	2.5	µg		Choline, total	18.7	mg
Vitamin C, total	89.2	mg		Tryptophan	0.033	g
Thiamin	0.071	mg		Threonine	0.088	g
Riboflavin	0.117	mg		Isoleucine	0.079	g
Niacin	0.639	mg		Leucine	0.129	g
Pantothenic acid	0.573	mg		Lysine	0.135	g
Vitamin B-6	0.175	mg		Methionine	0.038	g
Folate, total	63	µg		Cystine	0.028	g
Folic acid	0	µg		Phenylalanine	0.117	g
Folate, food	63	µg		Tyrosine	0.05	g
Folate, DFE	63	µg		Valine	0.125	g
Betaine	0.1	mg		Arginine	0.191	g
Vitamin B-12	0	µg		Histidine	0.059	g
Vitamin B-12	0	µg		Alanine	0.104	g
Vitamin A, RAE	31	µg		Aspartic acid	0.325	g
Retinol	0	µg		Glutamic acid	0.542	g
Carotene, beta	361	µg		Glycine	0.089	g
				Proline	0.11	g
				Serine	0.121	g

Table 6. Chemical composition of cauliflower (*Cauliflower, Usda.Gov*, n.d.)

Name	Amount	Unit		Name	Amount	Unit
Water	92.1	g		Fatty acids, total trans-polyenoic	0	g
Energy	25	kcal		Cholesterol	0	mg
Energy	104	kJ		Tryptophan	0.02	g
Protein	1.92	g		Threonine	0.076	g
Total lipid (fat)	0.28	g		Isoleucine	0.071	g
Ash	0.76	g		Leucine	0.106	g
Carbohydrate, by difference	4.97	g		Lysine	0.217	g
Fiber, total dietary	2	g		Methionine	0.02	g
Total Sugars	1.91	g		Cystine	0.02	g
Sucrose	0	g		Phenylalanine	0.065	g
Glucose	0.94	g		Tyrosine	0.051	g
Fructose	0.97	g		Valine	0.125	g
Lactose	0	g		Arginine	0.086	g
Maltose	0	g		Histidine	0.056	g
Galactose	0	g		Alanine	0.116	g
Calcium, Ca	22	mg		Aspartic acid	0.177	g
Iron, Fe	0.42	mg		Glutamic acid	0.257	g
Magnesium, Mg	15	mg		Glycine	0.071	g
Phosphorus, P	44	mg		Proline	0.071	g
Potassium, K	299	mg		Serine	0.086	g
Sodium, Na	30	mg		Alcohol, ethyl	0	g
Zinc, Zn	0.27	mg		Caffeine	0	mg
Copper, Cu	0.039	mg		Theobromine	0	mg
Manganese, Mn	0.155	mg		Choline, total	44.3	mg
Selenium, Se	0.6	µg		Vitamin B-12	0	µg
Fluoride, F	1	µg		Vitamin B-12, added	0	µg

Vitamin C, total ascorbic acid	48.2	mg		Vitamin A, RAE	0	µg
Thiamin	0.05	mg		Retinol	0	µg
Riboflavin	0.06	mg		Carotene, beta	0	µg
Niacin	0.507	mg		Carotene, alpha	0	µg
Pantothenic acid	0.667	mg		Cryptoxanthin, beta	0	µg
Vitamin B-6	0.184	mg		Vitamin A, IU	0	IU
Folate, total	57	µg		Lycopene	0	µg
Folic acid	0	µg		Lutein + zeaxanthin	1	µg
Folate, food	57	µg		Vitamin E (α-tocopherol)	0.08	mg
Folate, DFE	57	µg		Vitamin E, added	0	mg
Vitamin D(D2+D3)	0	IU		Tocopherol, beta	0	mg
Vitamin D(D2+D3)	0	µg		Tocopherol, gamma	0.2	mg
Vitamin K (phylloquinone)	15.5	µg		Tocopherol, delta	0	mg

Table 7. Chemical composition of carrot (*Carrots, Usda.Gov*, n.d.)

Name	Amount	Unit		Name	Amount	Unit
Water	88.3	g		Vitamin A, RAE	835	µg
Energy	41	kcal		Retinol	0	µg
Energy	173	kJ		Carotene, beta	8280	µg
Protein	0.93	g		Carotene, alpha	3480	µg
Total lipid (fat)	0.24	g		Cryptoxanthin, beta	0	µg
Ash	0.97	g		Vitamin A, IU	16700	IU
Carbohydrate	9.58	g		Lycopene	1	µg
Fiber, total dietary	2.8	g		Lutein + zeaxanthin	256	µg
Total Sugars	4.74	g		Vitamin E (α-tocopherol)	0.66	mg
Sucrose	3.59	g		Vitamin E, added	0	mg
Glucose	0.59	g		Tocopherol, beta	0.01	mg
Fructose	0.55	g		Tocopherol, gamma	0	mg
Lactose	0	g		Tocopherol, delta	0	mg
Maltose	0	g		Tocotrienol, alpha	0.01	mg
Galactose	0	g		Tocotrienol, beta	0	mg
Starch	1.43	g		Tocotrienol, gamma	0	mg
Calcium, Ca	33	mg		Tocotrienol, delta	0	mg
Iron, Fe	0.3	mg		Vitamin D (D2 + D3)	0	IU
Magnesium, Mg	12	mg		Vitamin D (D2 + D3)	0	µg
Phosphorus, P	35	mg		Vitamin K (phylloquinone)	13.2	µg

Potassium, K	320	mg		Vitamin K (Dihydrophylloquinone)	0	µg
Sodium, Na	69	mg		Fatty acids, total sat	0.032	g
Zinc, Zn	0.24	mg		Tryptophan	0.012	g
Copper, Cu	0.045	mg		Threonine	0.191	g
Manganese, Mn	0.143	mg		Isoleucine	0.077	g
Selenium, Se	0.1	µg		Leucine	0.102	g
Fluoride, F	3.2	µg		Lysine	0.101	g
Vitamin C, total	5.9	mg		Methionine	0.02	g
Thiamin	0.066	mg		Cystine	0.083	g
Riboflavin	0.058	mg		Phenylalanine	0.061	g
Niacin	0.983	mg		Tyrosine	0.043	g
Pantothenic acid	0.273	mg		Valine	0.069	g
Vitamin B-6	0.138	mg		Arginine	0.091	g
Folate, total	19	µg		Histidine	0.04	g
Folic acid	0	µg		Alanine	0.113	g
Folate, food	19	µg		Aspartic acid	0.19	g
Folate, DFE	19	µg		Glutamic acid	0.366	g
Choline, total	8.8	mg		Glycine	0.047	g
Betaine	0.4	mg		Proline	0.054	g
Vitamin B-12	0	µg		Serine	0.054	g

2. Aim of thesis

Freezing can have varied effects on the antioxidant properties and nutrient content of vegetables, with outcomes depending on the vegetable type and storage conditions. For instance, Çubukçu et al. (Çubukçu et al., 2019) found that freezing improved the antioxidant activity of broccoli but had detrimental effects on cauliflower. Meanwhile, in beetroots, freezing initially led to a decrease in total polyphenol content and antioxidant activity before in vitro digestion; however, frozen beetroots demonstrated a higher release and bioaccessibility of antioxidants (Dalmau et al., 2019). From another point of view, the findings of Kapusta-Duch et al. (Kapusta-Duch et al., 2019) revealed a negative impact of frozen storage on the antioxidant activities of broccoli and cauliflower, primarily due to a reduction in vitamin C and polyphenols. However, in cauliflower stored at −22 °C for up to 3 months, changes in β-carotene and carotenoids were minimal and only became statistically significant with more extended storage periods, such as 12 months. This suggests that while certain antioxidants, like vitamin C and polyphenols, decline more quickly in frozen storage, others remain relatively stable over shorter periods (Neri et al., 2020).

Also, in the point of packaging effects, Cauliflower stored in polyethylene low-density (PE-LD) and oriented polystyrene (OPS) packages reported a 5-6.5% decline in dry mass, a 69-80% decline in vitamin C, and a slight increase in antioxidant potential after 3 months of frozen storage. Notably, packaging type had no significant impact on nutrient levels or antioxidant activity. Additionally, total polyphenols in vegetables stored in PE-LD and OPS packages declined by 7-14% after freezing and 1 month of storage. However, further storage generally only led to significant changes compared to blanched samples. These findings suggest that while freezing affects certain nutrients initially, some may stabilise over extended frozen storage, with minimal impact from packaging material (Kapusta-Duch et al., 2017).

The complex chemical structure and diversity of phenolic compounds make it challenging to measure their total concentration accurately. Each compound may respond differently depending on the method applied, leading to limitations in quantification accuracy. As a result, no existing analytical approach is universally

recognised as optimal or comprehensive for determining the total levels of phenolic compounds (Wang & Weller, 2006).

In the present study, different cultivars of vegetables were stored under identical plastic packaging and freezing conditions, simulating common household practices, and the percentage of changes in antioxidant activities and total phenolic contents before and after freezing were analysed. This approach allowed us to assess how freezing impacts these properties consistently across different cultivars, providing insight into the stability of antioxidants and phenolic contents under controlled frozen storage.

3. Materials and Methods

3.1 Vegetables

Fresh samples collected from agricultural fields were used for investigation. The first step involves cleaning the fresh plant material to remove dirt, debris, and any surface contaminants that are essential for food safety and ensuring the produce is free from unwanted particles (Grzeszczuk et al., 2007; Lisiewska & Kmiecik, 1997). After cleaning, a bit of each was used for the first test and remained samples were labelled and stored in a freezer at -18 ˚C for the following observation.

For extraction samples, 10 mg of each chopped sample was mixed in 10 mL of 50 % (vol.) ethanol for 1 hour and continuously stirred at room temperature in a dark place. After that, extracts were filtered through a micropore paper filter of 2.2 µm size, and the filtrated samples were used to test antioxidant activity immediately (Dziedziński et al., 2020; Stankevičius & Jakobsone, 2010). After completing the antioxidant tests, the subsequent testing round is scheduled for the next three months. The samples, which are cut and chopped, will be kept frozen without thawing, and 10 mg from each sample, as before, will be used for extraction. We examined five distinct culinary plants, each represented by four unique cultivars, as detailed in Table 8. Although these products were received on different dates, all were stored frozen for a period of three months.

Table 8: Overview of Plants and Cultivars Examined in This Study

plant	Variety	Reference
Broccoli	Monaco	(Monaco - Variety Broccoli)
	Andersia	(Andersia - Variety Broccoli)
	Montop	(Montop - Variety Broccoli)
	Beany	(Beany - Variety Broccoli)
Cauliflower	David	(David - variety Cauliflower)
	Gohan	(Gohan - Variety Cauliflower)
	Almagro	(Almagro-Variety Cauliflower)
	Java	(JAVA - Variety Cauliflower)
Beetroot	Subeto	(Subeto - Variety Beetroot)
	Monty	(Monty - Variety Beetroot)
	Boro	(Boro - Variety Beetroot)
	Action	(Action - Variety Beetroot)
Parsley	Arat	(Arat - Variety Parsley Root)
	Eagle	(Eagle - Variety Parsley Root)
	Navajo	(Navajo - Variety Parsley Root)
	Comanche	(Comanche - Variety Parsley Root)
Carrot	Warmia	(Warmia - Variety Carrot)
	Muleta	(Muleta - Variety Carrot)
	Bangor	(Bangor - Variety Carrot)
	Komarno	(Komarno - Variety Carrot)

3.2 Determination of antioxidant activity (AOA)

To survey the antioxidant activity of samples, the DPPH and ABTS radical scavenging assay are standard methods for assessing antioxidant potential. These are stable free radicals that change colour when exposed to antioxidants. The degree of colour change reflects the antioxidant activity.

Differences Between ABTS and DPPH Assays:

- **Solubility**: The ABTS radical is soluble in both organic and aqueous solvents; this ability makes the ABTS assay more versatile than the DPPH assay that is used in organic solvents like methanol or ethanol.
- **Wavelength**: ABTS is measured at 734 nm, while DPPH is measured at 517 nm.
- **Sensitivity**: ABTS can be considered more sensitive to certain types of antioxidants, predominantly hydrophilic; on the other hand, DPPH tends to work better with lipophilic antioxidants (Sadeer et al., 2020).

Table 9 presents the fundamental differences between these methods

Table 9: Comparison of the tests used to measure antioxidant activity in this study: (Prior et al., 2005; Singleton et al., 1999)

Assay	Principle	Solubility	Reaction Time	Sensitivity	Primary Use
ABTS	Radical scavenging	Hydrophilic & Lipophilic	Fast	many antioxidants	Broadly applicable
DPPH	Radical scavenging	Lipophilic	Moderate	lipophilic antioxidants	Foods & simple antioxidants
TPC	Phenolic compound estimation	Hydrophilic & Lipophilic	Moderate	All phenolics, not all antioxidants	Phenolic-rich samples

3.2.1 DPPH procedure

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay first time was introduced by Marsden Blois, working at Stanford University in 1958 (Blois, 1958)

In the originating paper by Blois, it is recommended to use an automatic burette with a nitrogen atmosphere and aluminium foil cover to prevent stability of free radical activity, which may be reduced slowly and deteriorating just 2-4 per cent per week (Blois, 1958; Molyneux, 2004). DPPH 120μM solution was prepared with 95% ethanol. By micropipette, 10 μL of the extract was mixed with 195 μL of DPPH in 96-well microtiter plates. The reaction mixtures were incubated at room temperature for 30 minutes in a dark place. A Microplate Reader measured absorbance at 517 nm. The values are expressed as the means of triplicate analysis.

$$\textbf{Inhibition of DPPH percent} = \frac{\mathbf{Ac-As}}{\mathbf{Ac}} \times \mathbf{100}$$

where "Ac" means the absorbance of the control (10 μL of water instead of the sample) and "As" means the average absorbance of the sample. (Wong-Paz et al., 2014; Xu, 2017)

3.2.1.1 Calculation and Preparation of the required mass of DPPH

The molecular weight of DPPH = 394.32 g/mol, and the desired concentration is 120 μM. To calculate the mass:

mass (g)=concentration (M)×molecular weight (g/mol) ×volume (L)

For 120 μM (or 120 × 10^{-6} M) in a final volume of 100 mL (0.1 L):

mass=120×10^{-6} M×394.32 g/mol×0.1 L=0.00473 g=4.73 mg

Weigh out 4.73 mg of DPPH on an analytical balance and transfer the DPPH into a 100 mL volumetric flask; now, add 95% ethanol to the flask until it reaches the 100 mL mark. Cap the flask and shake until the DPPH fully dissolves (Brand-Williams et al., 1995).

3.2.1.2 Calculating the fluctuation of DPPH before and after freezing

To calculate the fluctuation of DPPH after three months, the results obtained from triplicate samples and controls were analysed initially. Now these values are applied to this formula to determine the percentage of change:

$$\textbf{Percentage of decrease} = \frac{\textbf{Before freezing} - \textbf{After freezing}}{\textbf{Before freezing}} \times \mathbf{100}$$

3.2.2 ABTS procedure:

In a 96-well microtitre plate, 10 µl of the test substance or control (ethanol) was added to 195 µL of diluted stable ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical solution. After 30-minute at room temperature, the decrease in absorbance at 734 nm was measured against an ethanol blank. This method provides a quick evaluation of a substance's ability to neutralise free radicals. The values are expressed as the means of triplicate analysis (Didunyemi et al., 2020; Re et al., 1999; Tian & Schaich, 2013) .

$$\textbf{percentage Inhibition} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \times \mathbf{100}$$

where “Ac” means the absorbance of the control (10 µL of water instead of the sample) and “As” means the average absorbance of the sample.

3.2.2.1 Preparation of the ABTS Stock Solution:

Mix 7 mM of ABTS (about 38.4 mg per 10 mL of distilled water) with 2.45 mM of potassium persulfate solution (about 6.6 mg per 10 mL of distilled water) in a 1:1 ratio. Store the mixture in the dark at room temperature for 12-16 hours. This step allows the ABTS to react with potassium persulfate to form the ABTS radical cation ($ABTS^{+}$), which gives a green-blue colour. Dilute the solution with ethanol to an absorbance of about 0.70 at 734 nm for use in antioxidant assays. This absorbance is typically adjusted based on spectrophotometer calibration (Re et al., 1999a).

3.2.2.2 Calculating the fluctuation of ABTS before and after freezing

The results obtained from triplicate samples and controls were initially analysed and are now applied to calculate the fluctuation of ABTS after three months. These values are now applied to this formula to determine the percentage of change:

$$\textbf{Percentage of decrease} = \frac{\textbf{Before freezing} - \textbf{After freezing}}{\textbf{Before freezing}} \times \mathbf{100}$$

3.3 Determination of total phenolic content (TPC) assay

TPC is not a direct measure of antioxidant capacity but rather an estimation of phenolic compounds, a significant group of antioxidants. The Folin-Ciocalteu reagent reacts with phenolic groups in a sample, leading to a colour change that can be quantified.

3.3.1 TPC procedure

The total phenolic content (TPC) of the extracts was determined using a colorimetric assay. The protocol was adapted for microplate analysis with small volumes. Specifically, each well combined 20 µL of extract (1 mg/mL) with 20 µL of Folin-Ciocalteu (FC) reagent. After 5 minutes, 20 µL of sodium carbonate solution (0.01 M) was added, followed by another 5-minute incubation. The mixture was then diluted with 125 µL of distilled water and incubated at room temperature for 80 minutes. Finally, absorbance was measured at 790 nm using a spectrophotometer microplate reader. According to a GA standard curve, the TPC measurements were expressed as milligrams of gallic acid equivalents per gram of dry plant material (mg GAE/g), and these values are expressed as the means of triplicate. (Wong-Paz et al., 2014)

$$\textbf{percentage Inhibition} = \frac{\mathbf{Ac-As}}{\mathbf{Ac}} \times \mathbf{100}$$

where "Ac" means the absorbance of the control (20 µL of water instead of the sample) and "As" means the average absorbance of the sample.

After determining the percentage inhibition, the results were further analysed using the standard curve shown in **Graph 1** and this formula to calculate the phenolic content in terms of mg GAE/g:

$$\mathbf{Y = MX + C}$$

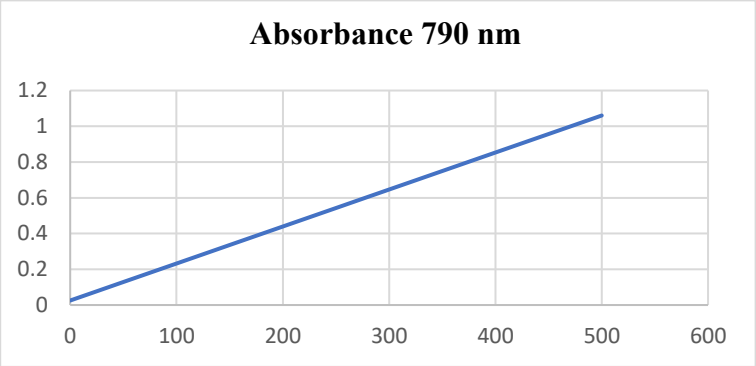
Y: absorbance

M: Slope

X: Concentration

C: Y-intercept

Graph 1: the standard curve of Gallic acid



3.3.2 Calculating the fluctuation of TPC before and after freezing

To calculate the fluctuation of TPC after three months, the results obtained from triplicate samples and controls were analysed initially. These values are now applied to this formula to determine the percentage of change:

$$\textbf{Percentage of decrease} = \frac{\textbf{Before freezing} - \textbf{After freezing}}{\textbf{Before freezing}} \times \mathbf{100}$$

4. Results and discussion

The data presented in Table 13 reveal significant fluctuations in antioxidant activities (AOA) among all groups of plants and cultivars after three months of freezing. These variations were assessed using DPPH and ABTS assays, which showed that a specific cultivar exhibited the least decrease in AOA in each plant group. In broccoli, the cultivar *Montop* demonstrated reductions of 6.63% (DPPH) and 17.41% (ABTS). Similarly, in cauliflower, the cultivar *Gohan* showed decreases of only 1.18% (DPPH) and 4.84% (ABTS). For beetroot, the cultivar *Boro* recorded reductions of 7.79% (DPPH) and 9.99% (ABTS), while parsley's cultivar *Eagle* exhibited decreases of 4.07% (DPPH) and a remarkably low 0.13% (ABTS). In carrots, the cultivar *Bangor* displayed reductions of 7.04% (DPPH) and 60.57% (ABTS), indicating a notable divergence between the two assays. Interestingly, the ABTS assay did not confirm the DPPH results for this cultivar of carrots. By the way, Carrots demonstrated the highest pre-freezing AOA levels, as highlighted in Tables 10 and 11, underscoring their superior antioxidant properties compared to other groups.

Fluctuations in total phenolic content (TPC), as detailed in Table 12, exhibited similar ranges among cultivars within the same plant group, except for parsley, where significant differences were observed. Also, the beetroot group showed the most phenolic compounds among the other groups. The maximum TPC reduction was recorded in the carrot *Komarno* (97.46%), while the parsley cultivar *Comanche* displayed a minimal reduction (17.65%).

A comparative analysis of pre- and post-freezing results indicated a significant impact of freezing on both AOA and TPC, particularly in specific cultivars. This variation underscores the differing levels of resistance to freezing-induced stress among cultivars. The minimal changes observed in particular cultivars suggest inherent resilience, which may be attributed to variations in cellular structure, biochemical composition, or antioxidant defence mechanisms. Such resilience makes these cultivars promising candidates for prolonged storage or export under freezing conditions, as they can better retain nutritional and functional properties.

Notably, the frozen samples in this study were tested immediately after freezing without undergoing a complete thawing process. This methodological approach aimed to capture the immediate state of biochemical components, minimising potential degradation that could occur during thawing. As a result, the observed data reflect the direct effects of freezing, free from the additional stresses imposed by thawing. This approach may have influenced the results for sensitive compounds, such as antioxidants and phenolic compounds.

The variability in freezing resilience observed across cultivars emphasises the importance of selecting varieties based on their ability to withstand freezing stress for long-term storage. Cultivar-specific resistance suggests a valuable avenue for breeding programs that enhance freezing tolerance in vegetable crops. These findings provide critical insights into optimising vegetable storage practices, mainly for export and extended preservation, and highlight the potential for further research to improve postharvest stability in frozen produce.

A study conducted by (Papuc et al., 2016) , demonstrated that fermented parsley juice exhibits a DPPH radical scavenging activity of 79.45%, indicating strong antioxidant potential and a Total Phenolic Content (TPC) of 14.87 mg GAE/g. Research by (Latinović et al., 2024), reported a 12% decrease in TPC after parsley underwent freeze-drying, underscoring the significant impact of processing on its phenolic composition and antioxidant properties. Interestingly, parsley subjected to open-air drying at room temperature showed a 5% increase in TPC, demonstrating that less intensive drying methods may help preserve or enhance phenolic content. These findings highlight the variability in TPC outcomes depending on the processing technique used and without any storage period.

Our study analysing TPC after three months of freezing showed a minimum of 17.65% in Comanche and a maximum change in Eagle with 80.2 %, which shows this fluctuation after a prolonged storage period.

A study by (Yasaminshirazi et al., 2021) on beetroot genotypes grown at the Kleinhohenheim research station during 2017 and 2018 reported Total Phenolic Content (TPC) values ranging from 201 to 612 mg GAE/g per dry weight. These findings align closely with the results from our study, where TPC values of 158.48, 350.11, 156.71, and 295.04 mg GAE/g were observed for the Subeto, Monty, Boro, and

Action genotypes, respectively. Overall, beetroot exhibited the highest phenolic compound content among all the plants evaluated in our research, highlighting its superior potential as a source of phenolic antioxidants.

According to (Vinson et al., 1998), the polyphenol content in broccoli was approximately 50% higher than that in cauliflower. This highlights broccoli's richer phenolic profile. In our study, this comparison revealed a higher amount of phenolic contents, as evidenced by TPC analysis. Furthermore, in a related survey, broccoli demonstrated significantly greater antioxidant activity compared to cauliflower, highlighting its superior antioxidant potential. A study by (Sikora et al., 2008), reported that freezing reduced broccoli's Total Phenolic Content (TPC) by approximately 26%, accompanied by a 12% decrease in its antioxidant activity. In our study, the changes varied significantly across genotypes. For the broccoli variety Montop, we observed minimal reductions of 6.63% in DPPH and 42.94% in TPC. In contrast, the Beany genotype exhibited the highest fluctuations, with a 71.84% decrease in antioxidant activity and an 88.57% reduction in TPC. These results highlight genotype-specific responses to freezing, suggesting that the preservation of bioactive compounds may depend heavily on the specific variety. These findings underscore the potential impact of freezing on bioactive compounds and antioxidant capacity in broccoli.

In a study by (Puupponen-Pimiä et al., 2003), observed that industrial processing, including blanching, immediate freezing at -40°C, and storage at -20°C, resulted in a 20% reduction in (AOA) and a 15% decrease in (TPC) for cauliflower. Interestingly, broccoli showed only minor fluctuations in these parameters under the same conditions, indicating more excellent stability of its bioactive compounds during processing and storage.

In our study of cauliflower, the DPPH scavenging activity decreased by an average of 20%, indicating a moderate reduction in its antioxidant potential. However, the average Total Phenolic Content (TPC) among all varieties of cauliflower showed a sharp decline of approximately 60%, underscoring cauliflower's sensitivity to preserving phenolic compounds during processing. These findings highlight the susceptibility of cauliflower to significant losses in bioactive compounds, particularly phenolics, under certain conditions.

In Carrots (Danesi & Bordoni, 2008), observed that the antioxidant activity was decreased after freezing under home conditions at -18°C. However, in a separate study by (Patras et al., 2011), freezing carrots at -30°C revealed no significant changes in antioxidant activity.

In our study, freezing at -18°C for three months resulted in decreases in DPPH scavenging activity by approximately 48.35%, 64.41%, 7.04%, and 20.69% for the Warmia, Muleta, Bangor, and Komarno varieties, respectively. However, the changes in Total Phenolic Content (TPC) were much more pronounced, showing a devastating decline across all varieties. These findings emphasise the significant impact of freezing on both antioxidant activity and phenolic content, with the extent of the changes varying by genotype

In a study on freezing and packaging vegetables by (Kapusta-Duch et al., 2019b) , it was observed that after 3 months of frozen storage, the type of storage container, sealed-oriented polystyrene (OPS) or low-density polyethylene (PE-LD), did not significantly impact the antioxidant activity. This aligns with the fact that storage container type did not appear to influence the extent of changes in antioxidant properties during freezing.

5. Conclusive remarks

This study underscores the complex interplay between freezing conditions and the biochemical stability of vegetables, offering critical insights into the impacts of freezing on antioxidant activity (AOA) and total phenolic content (TPC) across a wide range of cultivars. While freezing is an essential tool for extending shelf life, its effects on nutritional quality and bioactive compounds are not uniform. The results highlight significant variability among cultivars, with specific ones demonstrating remarkable resilience. Cultivars such as Montop (broccoli), Gohan (cauliflower), Boro (beetroot), Eagle (parsley), and Bangor (carrot) retained higher levels of AOA and TPC, making them excellent candidates for long-term storage and export. This resilience not only

enhances their market value but also reduces food waste by ensuring prolonged nutritional and functional quality.

The findings also emphasize the critical role of cultivar-specific traits in determining freezing tolerance. This variability provides a foundation for targeted breeding programs aimed at enhancing the post-freezing stability of vegetables, thereby supporting sustainable food systems, and addressing the growing demand for high-quality frozen produce. By identifying cultivars with strong resistance to freezing-induced stress, this research contributes to optimizing frozen vegetable supply chains, enhancing their economic and nutritional potential.

A key methodological strength of this study was the immediate testing of frozen samples without full thawing. This approach captured the direct biochemical impacts of freezing, minimizing confounding factors introduced by thawing. Such a perspective offers a clearer understanding of the state of bioactive compounds post-freezing and provides actionable insights for improving storage and handling practices in the frozen food industry.

Additionally, comparative analyses revealed that pre-freezing AOA and TPC levels strongly influence post-freezing outcomes, with beetroot emerging as a phenolic compound-rich crop and carrots exhibiting assay-specific variability. These results align with existing literature while highlighting genotype-specific differences that are essential for tailoring freezing practices to individual cultivars.

In conclusion, this study not only advances our understanding of the biochemical effects of freezing but also opens new avenues for innovation in postharvest technology. By leveraging the inherent resilience of certain cultivars, it is possible to optimize preservation methods, improve food security, and reduce nutritional losses in frozen produce. Future research should explore deeper into the genetic, cellular, and biochemical mechanisms underlying freezing tolerance to drive advancements in breeding and postharvest technologies. These efforts will play a pivotal role in addressing global challenges in food preservation, sustainability, and nutrition.

Table 10: Results of antioxidant activities in the different cultivars in the DPPH test before and after freezing

Plants	Variety	DPPH radical scavenging activity (%) Before freezing	DPPH radical scavenging activity (%) After freezing
Broccoli	Monaco	**24.07**	**20.11**
	Andersia	**49.68**	**16.83**
	Montop	**35.89**	**33.51**
	Beany	**25.25**	**7.11**
Cauliflower	David	**4.13**	**3.64**
	Gohan	**27.71**	**27.38**
	Almagro	**5.05**	**3.94**
	Java	**11.42**	**6.52**
Beetroot	Subeto	**33.65**	**20.11**
	Monty	**24.59**	**16.83**
	Boro	**36.34**	**33.51**
	Action	**16.34**	**7.11**
Parsley	Arat	**11.28**	**2.73**
	Eagle	**17.62**	**16.90**
	Navajo	**14.41**	**13.46**
	Comanche	**22.72**	**10.04**
Carrot	Warmia	**63.45**	**32.77**
	Muleta	**47.14**	**16.78**
	Bangor	**23.27**	**21.63**
	Komarno	**29.54**	**23.42**

Table 11: Results of antioxidant activities in the different cultivars in the ABTS test before and after freezing

Plants	Variety	ABTS radical scavenging activity (%) Before freezing	ABTS radical scavenging activity (%) After freezing
Broccoli	Monaco	46.78	31.16
	Andersia	53.59	29.77
	Montop	45.02	37.18
	Beany	46.82	21.71
Cauliflower	David	38.08	20.46
	Gohan	46.62	44.37
	Almagro	40.87	38.55
	Java	45.82	23.33
Beetroot	Subeto	50.96	48.20
	Monty	28.58	25.56
	Boro	24.97	22.48
	Action	33.23	29.12
Parsley	Arat	24.21	15.43
	Eagle	32.45	32.41
	Navajo	30.89	20.75
	Comanche	42.05	41.12
Carrot	Warmia	83.38	43.76
	Muleta	83.37	37.29
	Bangor	84.73	33.41
	Komarno	84.47	32.00

Table 12: Total phenolic contents in the different cultivars before and after freezing

Plants	Variety	Avg mg GA/g Before freezing	Avg mg GA/g After freezing
Broccoli	Monaco	**58.81**	**17.10**
	Andersia	**92.95**	**18.23**
	Montop	**59.61**	**34.01**
	Beany	**55.26**	**6.31**
Cauliflower	David	**3.41**	**1.32**
	Gohan	**5.83**	**2.45**
	Almagro	**6.95**	**1.48**
	Java	**17.10**	**3.57**
Beetroot	Subeto	**158.48**	**48.34**
	Monty	**350.11**	**33.04**
	Boro	**156.71**	**16.29**
	Action	**295.04**	**21.45**
Parsley	Arat	**19.84**	**9.21**
	Eagle	**81.83**	**16.13**
	Navajo	**22.57**	**15.49**
	Comanche	**45.60**	**37.55**
Carrot	Warmia	**71.37**	**26.76**
	Muleta	**65.57**	**8.24**
	Bangor	**53.81**	**5.51**
	Komarno	**83.93**	**2.12**

Table 13: Fluctuations among all antioxidant activities and amount of total phenolic contents in the different cultivars before and after freezing

plant	Variety	DPPH%	ABTS%	TPC%
Broccoli	Monaco	16.48	33.37	70.91
	Andersia	66.12	44.44	80.38
	Montop	6.63	17.41	42.94
	Beany	71.84	53.62	88.57
Cauliflower	David	11.84	46.26	61.26
	Gohan	1.18	4.84	57.97
	Almagro	21.99	5.69	78.66
	Java	42.91	49.08	79.08
Beetroot	Subeto	40.25	5.41	69.49
	Monty	31.58	10.55	90.56
	Boro	7.79	9.99	89.59
	Action	56.49	12.37	92.72
Parsley	Arat	75.80	36.26	53.56
	Eagle	4.07	0.13	80.28
	Navajo	6.57	32.84	31.37
	Comanche	55.79	2.22	17.65
Carrot	Warmia	48.35	47.51	62.49
	Muleta	64.41	55.28	87.42
	Bangor	7.04	60.57	89.76
	Komarno	20.69	62.11	97.46

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