To my dad

UNIVERSITÀ DI FOGGIA
Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente

Doctoral Thesis in
Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region
– XXVII cycle –

Optimizing storage conditions for minimally processed rocket leaves: effect of temperature and gas conditions on aromatic profile, sensorial and nutritional quality

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***Doctorate Course in 'Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region'* – XXVII cycle –**

Doctoral thesis on **'Optimizing storage conditions for minimally processed rocket leaves: effect of temperature and gas conditions on aromatic profile, sensorial and nutritional quality'**, discussed at the Università di Foggia, May 25 , 2015

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Contents

Abstract

'Rocket' is a collective name to indicate many species of green leaves belonging to Brassicacea family and characterized by a tipical pungent taste. Although the great variability and number of rocket species, *Eruca sativa* L. and *Diplotaxis tenuifolia* L. are the most common species, they are a source of a wide range of phytonutrients, such as provitamin A, vitamin C, flavonoids, glucosinolates, fiber, potassium and sulfur. Beneficial effects have been associated to the consumption of rocket due to the employment/ingestion of nutrient compounds characterized by diuretic, anti-inflammatory, stimulant, depurative, hepatoprotective and stomathic activities. Rocket leaves are very appreciated in Europe for their typical taste and texture and are manly consumed raw in salads. Due to the increased e consumption in the recent years, rocket it's mainly sold as a ready-to-eat product, which has been washed and packaged. The shelf-life of ready-to-eat rocket is principally limited by the storage conditions and processing operations, which may accelerate the degradation process, inducing wilting, discoloration, loss of nutritional properties and sensorial attributes as aroma and flavor. In this study was investigated the effect of several factors on the shelf-life of ready-to-eat rocket leaves in order to optimize the storage conditions through a correct management of the temperature and the gas composition inside the packaging. The effect of temperature on the degradation kinetics of sensorial, physical and chemical attributes of rocket leaves was studied. Weibull model was compared to conventional first-order model to describe quality changes over time in function of the temperature. Weibull model showed an higher ability to fit experimental data compared to conventional first-order models,

allowing to accurately describe shape and slope of the degradation curves. Obtained results also permitted to establish the most limiting factors for shelf-life of rocket leaves, which was therefore estimated in term of appearance score and ascorbic acid. As second partial objective different concentrations of O_2 (0.5, 3 and 6 kPa) and CO_2 (5, 10 and 20 kPa) were independently tested to evaluate their potential beneficial or negative effects on qualitative attributes of fresh rocket during storage. Generally different oxygen concentrations did not had a high impact on quality of rocket leaves, resulting in a slightly high sensorial quality for the leaves stored under 3 and 6 kPa O_2, whereas O_2 concentrations below 0.5 kPa negatively affected the shelf-life of rocket leaves. On the other hand, the effects of high CO_2 were much more evident than those of low oxygen, indicating that the visual appearance was better preserved in presence of CO_2 compared to the storage in air. Despite this positive effect on appearance a strong production of off-odors was observed for rocket stored with 20 kPa CO_2, whereas level of 10 kPa CO_2 did not induced any off-odor development. In conclusion results of the experiments on gas composition suggested that extreme gas concentrations with oxygen level of 0.5 kPa O_2 and accumulation of CO_2 up to 20 kPa should be avoided, whereas the addition of 10 kPa CO_2 to the air preserved the quality of the rocke leaves. The effect of the temperature on rocket leaves packaged in passive modified atmosphere was also evaluated. Degradation kinetics of the quality attributes on fresh-cut rocket were modeled in isothermal and non isothermal conditions in order to improve the management of the logistic chain according to the product thermal history. In particular, the cumulative form of the Weibull equation and a log-logistic model

were, respectively, used to fit the experimental data over time and to study the temperature dependence of the degradation rates for several sensorial, chemical and physical attributes. Moreover, since MAP beneficial effects can be lost if anaerobic conditions develop inside the packaging due to an improper packaging management, volatile changes in MAP stored rocket leaves were analyzed in isothermal (5°C) and non isothermal conditions, by homogenizing the samples, and therefore simulating the chewing process. Storage in MAP had a beneficial effect on the quality of rocket leaves preserving the freshness of the produce in terms of visual quality and retention of the typical odor. However, at the last days of storage an increased production of dimethyl sulfide and acetaldehyde was observed, which could respectively confer the typical sulfurous and ethereal odor to the produce. Moreover, temperature fluctuation induced changes in the volatile profile compared to rocket stored in isothermal conditions which persisted even when the cold chain control was restored. The last experiment was aimed to monitor the volatiles directly accumulated in the headspace of the packages to evaluate the effect of gas composition and temperature on the compounds responsible of the perceived odor after the opening of the package. Rocket leaves were packed and stored at 0, 5 and 15°C; additionally at 5°C a control in air was also included to test the effect of gas composition on volatile profile. Temperature played an important role by accelerating the degradation rate of the rocket leaves inducing the development of off-odors. At 15°C temperature lipid derivatives and sulphurous compounds were in fact produced in much higher quantity than at low temperatures. The typical aroma was best preserved at 0°C, in which no degradation process was observed in the last

days of storage. The effect of the MAP conditions compared to the control in air, was much lower than the effect of the temperature, with an higher perception of off-odors for MAP stored samples than for samples stored in air. All the results contributed to increase the knowledge on degradation reactions occurring during storage of rocket leaves under different temperature and gas conditions, providing important tools to improve the management of the critical control points and quality maintenance during the entire production chain.

Part one - General

1.1 Botanical taxonomy of rocket

'Rocket' is a collective name to indicate many species of green leaves belonging to Brassicacea family and characterized by a tipical pungent taste. The Brassicaceae family actually includes 3,709 species and 338 genera (Rich, 1991; Warwick et al., 2006, 2009). The rocket origins are located in the Mediterranean and in the Near East where the use of a common name is probably originated from the Latin speaking Roman citizens who settled in these regions (Hall et al, 2012a). Common names, including rughetta, rucola, roquette and others, perhaps descended from the Latin word '*roc*', which means 'harsh' or 'rough' (Pignone, 1997). Figure1 describes the botanical classification of its large and economically important family.

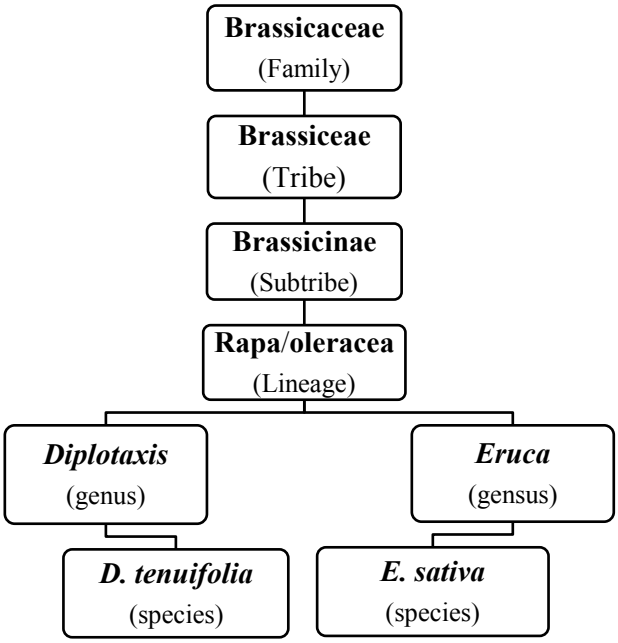

Figure 1 Botanical taxonomy of rocket species (from Hall et al., 2012a).

The common forms of commercially cultivated rocket are two, perennial and annual species, which are known as perennial wall rocket or wild rocket (*Diplotaxis tenuifolia* (L.) DC.) and annual garden rocket or salad rocket (*Eruca sativa* Mill.) (Hall et al., 2012a; Cavaiuolo et al., 2014).

Diplotaxis's name derives from the Greek words 'diplos', which means 'double', and 'taxis', which corresponded to 'row'; these words are referred to the arrangement of seeds inside the silique (Bianco, 1995). *Diplotaxis* spp. originated in the Mediterranean and in the Near East, and have a major center of diversity in the Western Mediterranean (Martínez-Laborde, 1997, Eschmann-Grupe et al., 2003). The plant is characterized by lengthened leaves and tap root (Pratap et al., 2009) and can reach 80 cm of height. The base of stem is woody and have retrorse hairs in the lower parts (Bianco, 1995). The typical leaf is fleshy, oblong and deeply lobed, while the apexes are sharp. The bright yellow flowers have a medium size and are characterized by four rounded petals, which can reach a length from 8 to 15 mm. They are organized in racemes with six tetradynamous stamens and have oblong or linear sepals (Pratap et a., 2009). The fruits have a cylindrical form, with a length ranged from 2 to 4 cm, and are semi-appressed along the stems (Pratap et al.2009, Hall et al., 2012a). Seeds are characterized by light brown color and have ellipsoidal form (De Leonardis et al., 1997; Martínez-Laborde, 1997). Rocket seeds germinate during the autumn, the plants form rosettes and tap roots. Seasonal conditions are important for the blooming, which can occur 28 days after germination (Kenigsbuch et al., 2009). The *Eruca* genus shows an high morphological variability; species can have round leaves or serrated leaves (Hall et

al., 2012a). This genus name derives from the Latin words '*uro*' or '*urere*', which means 'to burn' referring to the pungent taste of the leaves depended on the glucosinolates compounds (Bianco, 1995), the presence of which is established in the brassicaceae family.

Also this genus is native to the Mediterranean region (Pignone, 1997; Pratap et al., 2009). Annual garden rocket has been cultivated as a salad or spice in the Southern Europe and Central Asia for centuries (Ryder, 2002; Pratap et al., 2009).

Eruca rocket can grow to reach an height of 40 cm. It has a lyrate- pinnatifid leaf, which has an enlarged terminal lobe and smaller lateral lobes (Hall et al., 2012a). Leaves are arranged to form a rosette (Pratap et al.,2009). This species has a thin tap root and is characterized by a rigid unbranched stem with hairs. Leaves show differences depending by the position along the stem; they are pinnatifid at the apex of the plant and can be toothed or lobed with a dark green color and can reach a maximum length of 20 cm. The shape is variable; they can be smooth and round, lobed or serrated. Flowers are large, white or light yellow with purple venations (Hall et al., 2012a) and are composed by four petals (15-20 mm) with tetradynamous stamens (Bianco, 1995). Racemes are small and placed in terminal position. The siliques shape can be ovate-oblong or oblong and can reach the length of 3 cm (Bianco, 1995, Hall et al., 2012a). The typical seeds color is brown but can vary from yellow brown to olive green (De Leonardis et al., 1997).

Diplotaxis and *Eruca* spp. show similar morphology and a characteristic bitter taste depending by the presence of glucosinolates (Halkier et al., 2006).

1.2. Nutritional attributes

Although the great variability and number of rocket species, *Eruca sativa* L. and *Diplotaxis tenuifolia* L. are mostly used for human consumption (Cavaiuolo et al., 2014); they contain a wide range of phytonutrients, such as provitamin A, vitamin C, flavonoids, glucosinolates, fiber, potassium and sulfur. (Martínez-Sánchez et al., 2006a; Bennett et al.,2004). Rocket generates a low amount of calories (Cavaiuolo et al., 2014) and its leaves are manly consumed raw in salad. Seeds of *Eruca sativa* species are commonly used as lubrificant and to produce soap with the extract oil (Miyazawa et al., 2002).

Beneficial effects have been associated to the consumption of rocket due to the employment/ingestion of nutrient compounds characterized by diuretic, anti-inflammatory, stimulant, depurative, hepatoprotective and stomathic activities. Chemical analysis have shown an high content of health-promoting compounds in leaves and seeds, such as antioxidants and glucosinolates, which are known to prevent the cancer and have pharmaceutical activity (Cavaiuolo et al., 2014; Barillari et al., 2005; De Feo et al., 1995). Epidemiological studies have shown that the introduction of vegetables rich in glucosinolates in the diet (Bjorkman et al., 2011; Cohen et al., 2000; Traka et al., 2009; Verkerk et al., 2009) can reduce the incidence of some kind of cancer, such as colon (van Poppel et al., 1999; Seow et al., 2002), bladder (Bhattacharya et al., 2010), lung (London et al., 2000) ,and potentially breast (Fowke et al., 2003) and prostate cancers (Cohen et al., 2000; Kirsh et al., 2007)

1.2.1 Antioxidant compounds and metabolites

Rocket contains high levels of secondary metabolites, which are involved in the protection of tissue cells showing a free-radical scavenging activity. In response to oxidative stress and senescence degenerative processes, flowers and leaves produce antioxidant derivates, which include a wide range of different biomolecules.

1.2.1.1 Carotenoids

Carotenoids are natural fat-soluble pigments found in many fruits and vegetables. As pigment involved in the primary metabolism, they can absorb light energy in the wavelength range of 400-500 nm; these absorption bands confer to carotenoids a characteristic orange color. In plant, they act as accessory pigments able to transfer the absorbed light to the chlorophyll pigments involved in the photosynthesis process. It is also known their important role in the photoprotection of cells due to the absorption and release of excess absorbed energy accumulated in the photosynthetic membrane. On the other hand, it is recognized that these pigments have beneficial effects on the human health, they can prevent some kind of cancers, cardiovascular diseases and eye disorders (Nunes et al., 2003). Carotenoids are tetraterpenes synthesized through the mevalonate pathway using acetil-CoA or metabolic intermediates of glycolysis. Most of the vitamin A in the human diet derives from carotenoids (Rao et al., 2007), precursors of the vitamin A are β-cryptoxanthin, α-carotene and β-carotene. Cavaiuolo et al. 2014 described β-carotene and lutein to be the most abundant carotenoids in rocket. Lutein belongs to a group of carotenoid, which include vitamin A non-precursore. β-carotene amount

was 7.01 ± 1.04 mg 100 g−1 FW in wild rock et and 7.96 ± 1.43 mg 100 g−1 FW in salad rocket, while the lutein amount was 5.82 ± 0.51 mg 100 g−1 FW in wild rocket and 7.44 ± 0.78 mg 100 g−1 FW in salad rocket (Bryan et al., 2010).

The total amounts of carotenoids for rocket leaves is about 120 µg/100g (USDA, National nutrient databes for standard reference, 2015) which is a significan amount if considering the raccomended dietary allowance of 9700-00 µg /day (Oregon State University, 2015)

1.2.1.2 Vitamin C

Ascorbic acid (AsA) is a water-soluble vitamin involved as co-factor in many essential metabolic reactions in the human body; human anabolism doesn't include biosynthetic enzymes for vitamin C, which must therefore be taken from food (Nishikimi et al., 1994). On the contrary plants can synthesize AsA through several biosynthetic pathways involving the D-mannose–L-galactose pathway (Wheeler et al., 1998), L-glucose pathway (Wolucka et al., 2003), D-galacturonate pathway (Agius et al., 2003), and myo-inositol pathway (Lorence et al., 2004). More than 90% of vitamin C can be introduce in the human diet consuming fruits and vegetables (Lee et al., 2000). Rocket contain an high content of vitamin C (Bennett et al., 2006). Martínez-Sánchez et al. (2006a) reported that vitamin C content of vitamin C in wild rocket was 50mg 100 g−1 fw. Usually, vitamin C is referred to ascorbic acid (AsA) and its oxidative derived, the L-dehydroascorbic acid (DHA); both of them have shown biological activity. Human intestinal cells can absorb and reduce DHA in AA, which then permeate into the blood (Wilson, 2002). Vitamin C

is involved in the protection of proteins, lipids and carbohydrates from the damage caused by free radicals and reactive oxygen species (Davey et al., 2000). The consumption of fruits and vegetables can be valuable in providing the correct amount of this substances in the human diet. There are more evidences about its important role in the prevention of scurvy, maintenance of healthy skin, gums and blood vessels (Nunes et al., 2003). Vitamin C has also important biological functions in the human body such as collagen formation, absorption of iron, reduction of plasma cholesterol levels, inhibition of nitrosoammine formation and enhancement of immune system (Lee et al., 2000)

1.2.1.3 Phenolic compounds

Phenolic compounds are a class of heterogeneous secondary metabolites, which contain a phenolic group with a common basic structure corresponded to a hydroxyl group bound to an aromatic ring. They show different role in the defense of plant; some of them are involved in the protection from the herbivorous and pathogen attacks, whereas they are produced to attract pollinators and animals to disperse the seeds and fruits. They can also inhibit the grown of competitive plant species in the surrounding area or confer mechanical support to the plant structure. Most of phenolic compounds are biosynthesized starting from the shikimate pathway, which produce aromatic amminoacid including phenylalanine, the most important intermediate and substrates for phenylalanine-ammonia-lyase (PAL), a key enzyme for the production of several phenolic compounds. This enzyme catalyze the conversion of phenylalanine in *trans*-cinnamic acid through the release of ammonia.

Phenolic compounds include the flavonoids, pigments synthesized through two separated pathways (malonic and shickimate), which can be subjected to oxidation to quinines (Havsteen et al., 2006). They have also antiviral, antiprotozoal and interestingly antimicrobial activity. Rocket leaves are a resource of flavonoids. Quercetin derivatives were the most abundant compounds in *D. tenuifolia* leaves, which show a total flavonoids content ranging from 4.68 to 19.81 g/kg. While Kampferol derivatives represent the primary group of phenols compounds in *E. sativa* leaves ranging from 8.47 to 26.0 g/kg which correspond to 77%–88% of total phenols (Cavaiuolo et al., 2014; Pasini et al., 2011).

1.2.2 Glucosinolates

Glucosinolates (GLSs) are bioactive molecules found principally in Brassicacea family. Several functions are reported about GLSs in plants: as secondary metabolites, they are involved in the defense of the plants against the attack of herbivores, pathogens (Björkman et al., 2011) and against fungal diseases or pest infestation (Blažević et al., 2009); they are also involved in the sulphur and nitrogen metabolism and in the growth regulation. The tissues disruption and cells breakage induce the loss of cell compartimentalisation and the release of glucosinolates from cell vacuole, which are converted by enzymatic reaction in isothiocyanates (ITCs), thiocyanates, nitriles and sulfates in the cell cytosol. These compounds, containing nitrogen (N) and mostly sulfur (S) atoms, are responsible for the pungent and sulphurous taste and the typical odor of Brassicaceae vegetables (Cavaiuolo et al, 2014). The enzymatic reaction is due to myrosinase, belonging to an hydrolase

family. Figure2 shows the mechanism of action of myrosinase and the conversion of GLSs in their reaction products.

It is reported that rocket leaves contain an high content of glucoraphanin (4-methylsulfinylbutyl-GLS) glucoerucin (4-methylthiobutyl-GLS) and dimeric 4-mercaptobutyl-GLS; seeds and roots contain principally glucoerucin, while flowers contain glucosativin (Cavaiuolo et al., 2014; D'antuono et al.,2008; Cataldi et al., 2007). The hydrolysis of glucoerucin produces erucin (4-methylthiobutyl isothiocyanate), the most abundant isothiocyanate found in rocket leaves (Miyazawa et al., 2002).

Also isothiocyanates as their precursors, play an important role in preventing higher animal degenerative diseases through the regulation of the carcinogen-metabolizing enzymes involved in the detoxification and protection against oxidative stress (Cavaiolo et al, 2014; Barillari et al., 2005; Vaughn et al., 2005).

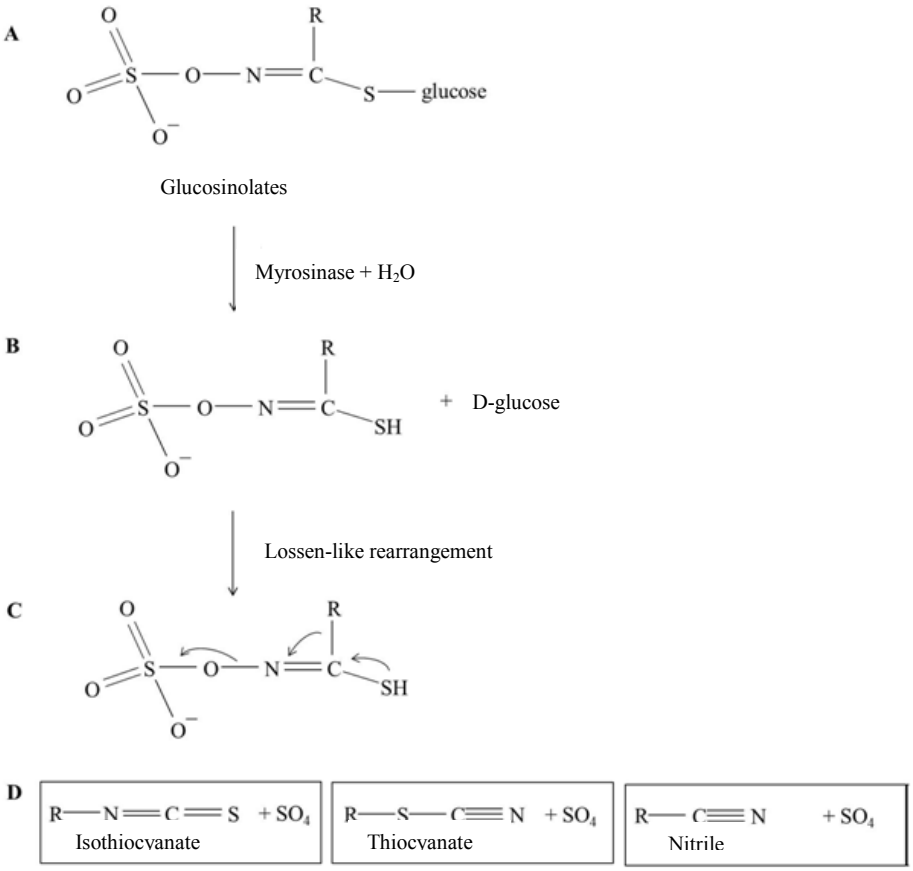

Figure 2 Mechanism of formation of ITC: Myrosinases hydrolizes glucosinolates (A) releasing aglicones D-glucose and water as reaction products (B). In the cell aglicones rearrange (C) releasing isothiocyanates, thiocyanate, nitriles and sulfate (D). (from Cavaiulo et al, 2014)

1.2.3 Volatiles organic compounds

Plants are adapted to interact with its abiotic and biotic environment producing volatile organic compounds (VOCs). These compounds are low molecular weight substances (less than 300 Da) with different structures, including hydrocarbons,

alcohols, aldehydes, ketones, ethers and esters which can be grouped in four classes corresponding to different metabolic origin: terpenoids, fatty acid derivatives including lipoxygenase pathway products, benzenoids/ phenylpropanoids and amino acid derivatives (Matarese et al., 2014). In plantw, the production of VOCs is related to diverse roles; they act as a chemical signal to interact with surrounding organisms to ensure the survival of the plant. Considering the whole plant, differences in VOCs profiles depending by the organs are associated to the different function to execute. Floral components produce volatiles able to attract the pollinators, in order to ensure reproductive and evolutionary success of the plant; while, leaf and root emit volatiles to defend the plant against the herbivores or pathogens behaving like toxins and deterrents or acting as a signal for herbivore enemies (Degenhardt et al., 2000; Reinhard et al., 2004; Kessler et al., 2001). Volatiles compounds are also responsible for the aroma of the fruits and vegetables. Brassicacea family are characterized by a wide range of volatile compounds, including isothiocyanates, terpenes, alcohols, esters, aldehydes and ketones (Taveira et al., 2009). Mechanical tissue damages, such as cutting, or light processing induces diverse biosynthetic pathways including the production of volatiles. In green-leaf volatiles pathway, C6-alcohols and -aldehydes, are produced from α-linolenic acid and linoleic acid through the lipoxygenase (LOX) action. Hexanal, *trans*-2-hexenal, cis-3-hexen-1-ol, *trans*-2-hexen-1-ol were reported to be responsible for green aroma in Austrian *Eruca sativa* salad (Arey et al., 1991; Kessler et al., 2002). Terpenoides are the most common group including monoterpenes and sesquiterpenes developed as a consequence of herbivorous

attack. Terpenes give an important contribute to define the aroma of fruit and vegetables, they are characterized by fruity note making the horticultural crops much appreciated. Their production can occur through two alternative pathways, mevalonate and non-mevalonate; as 'secondary metabolites', they are synthesized in plants as a result of selective mechanisms to increase the fitness of the species and provide a better adaptation to their ecological niche (Chen et al., 2011). Terpenes, such as their oxidised analogues (terpenoids), are important substances for the human health because they show apophlegmatic, antibacterial, antivirial, cholagogic, spasmolytic, pain-releasing and carcinogenesis-preventing properties (Bakkali et al., 2008; Kupska et al., 2014).

Also isothiocyanate are responsible of the aroma of rocket leaves. Miyazawa et al. (2002) found seven isothiocyanates in essential oil from leaves of *Eruca sativa* and identified butyl isothiocyanate, 3-butenylisothiocyanate, iso-hexylisothiocyanate, hexylisothiocyanate, 3-methyl-thiopropylisothiocyanate, the above mentioned 4-methylthiobutyl isothiocyanate and 5-methylthiopentylisothiocyanate, the second most abundant compound found in rocket leaves. Jirovetz et al. (2002) identified also tetrahydrothiophene, allyl-isothiocyanate, which are responsible for the horseradish aroma and 4-pentenyl isothiocyanate. Isothiocyanate widely contribuite to generate the sulphurous, pungent taste and the typical odor of rocket leaves.

1.3 Rocket as a fresh-cut produce

Rocket is very popular leafy vegetable in Europe and in other parts of the world appreciated for its typical taste and texture. The consumption of rocket as ready-to-eat vegetable is grown in the recent years, so rocket is available in the market as leaf salad. This product is commercially sold as whole leaves, which has a very small section on the petiole exposed to oxidation, allowing to obtain a high postharvest life (Egea-Gilabert at al., 2009; González et al., 2004). Rocket is sold washed or unwashed, packaged in trays wrapped in plastic film to avoid physical damage and to prevent wilting of leaves through loss of water (Løkke et al., 2013; Cantwell et al., 1998). Rocket leaves are normally harvested two or three times per cultivation and are primarily utilized for the fresh-cut processing or for the fresh market. For processing, leaves are washed and packaged in modified atmosphere (MAP) , while they are sold as loose product in the fresh market (Hall et al., 2013). Packaged rocket may be usually found in supermarkets stored in open refrigerated cabinets and show a typical shelf life ranging from 7 to 14 days (Martínez-Sánchez et al., 2006b; Martínez-Sánchez et al., 2006a; Nielsen et al., 2008). When stored in these open refrigeration systems, in the supermarket the produce may reach a temperature of ∼10°C (Koukounaras et al., 2007; Wagstaff et al., 2010). This condition is not optimal to reduce and retard the microbial growth and metabolic activity of the produce, but the consumers prefer to purchase produce exposed on open shelves (Hall et al., 2013).

Minimally processed vegetables are subjected to a faster physiological deterioration, biochemical changes and microbial degradation than intact produces

(O'Beirne et al., 2003), as a consequence of the enhanced metabolic activity, which usually results in the loss of typical color, texture and flavor (Kabir, 1994; Varoquaux et al., 1994). While conventional food-processing methods extend the shelf-life of products, minimally processed fruit are highly perishable and require refrigerated storage to ensure a prolonged shelf-life (Garcia et al., 2002). The shelf-life of ready-to-eat rocket leaves is principally limited by the storage conditions and processing operations, which may accelerate the degradation of the leaves, inducing wilting, discoloration, loss of nutritional properties, aroma and flavor (Koukounaras et al., 2009).

1.4 Production steps of fresh-cut rocket

Following is the description of a typical flow of operation for the production of minimally processed rocket leaves, which is schematically shown in Figure 3.

After the harvest, rocket leaves are transported in plastic boxes on wooden pallets at 5°C in refrigerated truck (long distance) or ambient temperature (short distance). In the factory, raw material is weighted and checked for qualitative and quantitative parameters, such as biological (Bremia, late blight, rot) and physiological (oxidation to the cutting point, micro deficiencies, soft rot, damage) alterations including dry necrosis.Other quality parameters are also evaluated as the presence of foreign bodies (inorganic, animals, flowers), to check the standard size (width leaves, stem length, length overall), the water content, and the temperature of product. Then it a lot number is automatically attributed to the lot related also with growing and

harvesting conditions for the product traceability. In this step product not matching minimum established standard is eliminated. The recommended room temperature during this operation is not higher than 15°C. Pre-cooling is done using forced-air

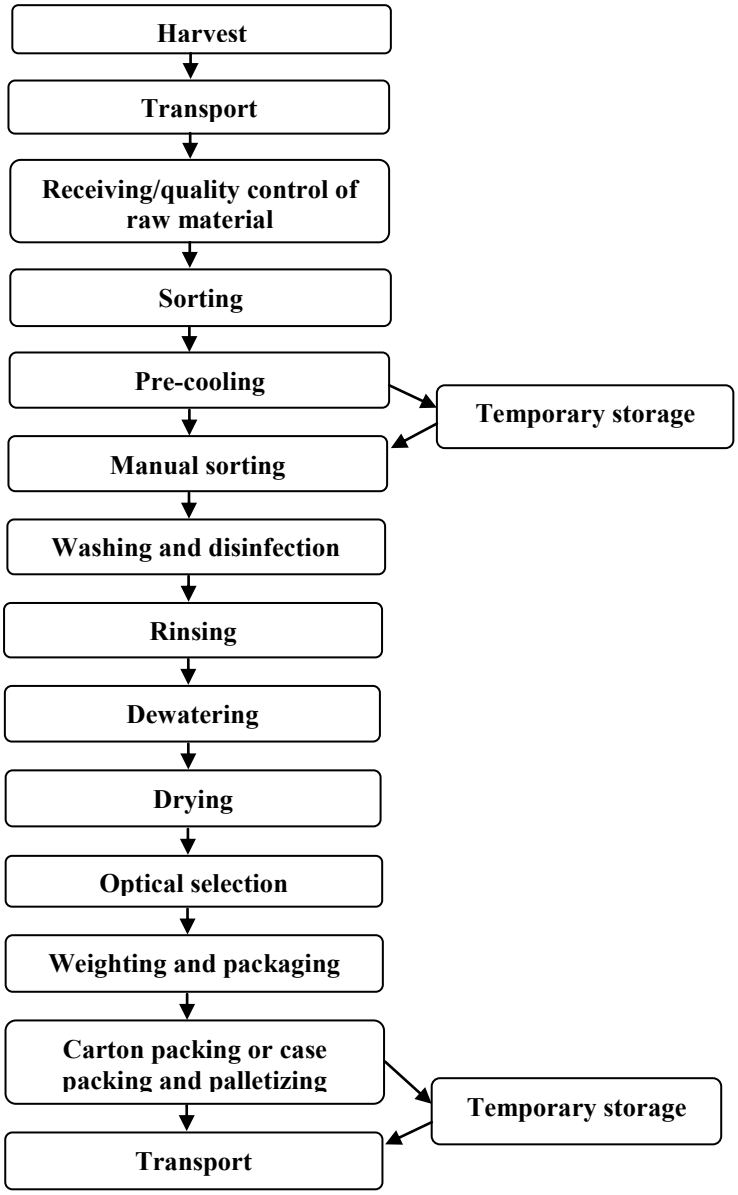

Figure 3. Flow diagram of the operation steps for minimally processed rocket leaves.

cooling by rapid movement of refrigerated air at a recommended temperature of 5°C. The processing start with a manual sorting characterized by the elimination of foreign bodies or non conforming materials. The recommended temperature room for processing operation is 10-12°C. Then leaves are washed with a bubble washing system using chlorinated water (50-100 ppm sodium hypochlorite), which has a temperature between 10-12°C (Photo 1), to eliminate the contaminants (biological and inorganic).

Photo 1. Example of bubble washing system used forwashing.

After this step, the produce is submitted to two-three cycles of rinsing using water at a temperature of 8-10°C, generally two bubble washing systems, the second with a rotating perforated drum, and a shower system. Dewatering is done using a vibrating sieve machine through the transport of the product along the line with the removal of the excess of water. Leaves are submitted to drying using forced-air

dryer tunnel, and in some cases with centrifugation, even though this system cause more mechanical damage to the leaves. Before packaging an optical control based on transparency, color and chlorophyll content allows to eliminate eventual foreign bodied or defected leaves not eliminated during the process. The eventual storage of semi-finished product is done in a cold room at 5°C; the produce is temporary stored in plastic box waiting to be packed for a time that may vary between 0-12 hours. The last production step correspond to weighting and packaging using a vertical/horizontal flow pack system (Photo 2), which introduce the product in plastic bag or tray in passive atmosphere, normally packaged in polypropylene. In Figure 2 a vertical flow pack machine is shown. The final product is finally checked for metal detection and final weight check. The packed leaves are stored at 10-12°C in cardboard box or case packing and palletized with automatic attribution of lot number related to raw material lot and day of production. Final product may be temporary stored in dedicated cold room for about 0-8 h, at a temperature of 2-8°C. Finally product is transported using refrigerate trucks and delivered to customers and / or distribution platforms.

Photo 2. A particular of a vertical flow-pack machine.

1.5 Environmental factors affecting quality of fresh-cut produce

Several factors affect the quality of fresh-cut produces, including the cultivar (genetic differences between species and reproductive cycles), the ripeness stage during processing, grown season, harvest number, plant development and storage atmosphere and temperature (Colás-Medá et al., 2015; Hall et al, 2013; Leverentz et al, 2001).

1.5.1 Pre-harvest factors

The growth characteristics of perennial wall rocket and annual garden rocket depend by the environmental conditions. Hall et al., (2012b) reported that the production of perennial wall rocket was optimal during the spring and summer,

while for annual garden rockets summer and winter gives the best quality. In another study, Hall et al. (2013) reported that shelf-life of perennial wall rocket leaves stored at 4 °C can range from 12 to 24 days after harvest for leaves grown in three different seasons (summer, spring and winter) and for 3 harvest times and that the summer was the period in which rocket had the longest shelf-life especially for rocket of the second harvest time.Rocket leaves grown and harvested in spring had a lowest shelf-life, of about 12 days, considering the second harvest time, whereas leaves grown in winter had intermediate shelf-life. For annual garden rocket leaves grown during the three seasons they reported a shelf life from 12 to 19 days with the longest shelf-life in rocket leaves grown in winter period, about 26 days for the second harvest time, which was reduced to 12 days in rocket grown in spring.

1.5.2 Temperature

Temperature is the most important factor affecting the quality of fresh-cut produce. Low temperature is essential to maintain an optimal product quality because it reduces several physiological activities, such as transpiration, which causes weight loss, and respiration rate. Respiration can induce chemical and enzymatic changes that may cause tissue softening, pigment loss, ripening and discoloration (Brosnan and Sun, 2001). Another important factor is that low temperature reduces the growth rate of spoilage microorganisms on surfaces of vegetable tissues. It is known that a cold chain management is the most important factor able to preserve the quality and safety of fresh-cut produce. The temperature of fresh produce should

be maintained below 5°C to reduce the proliferation of spoilage microorganisms and human pathogens (Rediersa et al., 2009; Ukuku et al., 2007). All horticultural crops should be kept at theri optimal temperature of storage, with an accepted fluctuation within ±1°C. Storage temperature below the optimal values can induce chilling injury, which can determine the collaps of the tissue, browning, pitting, off-flavors, increased incidence of moulds and decay, water soaked areas; while storage temperature above the optimal value increase the respiration rate of the produce and accelerate the metabolic activity causing a reduction of the shelf-life and rapid loss of quality. Temperature abuse determine the loss of functionality of cells causing heat injury, which are visible as bleaching, surface scalding, desiccation (Kader, 2002a).

The storage temperature is the principal factor to maintain the quality of rocket leaves, prolong their shelf life (Koukounaras et al., 2007; Watada et al., 1996) and preserving visual appearance. Intact rocket leaves can be stored at 0°C (Cantwell, 2001); mechanical damages, or morphological defects influence negatively the visual quality of the product; leaves dehydration and loss of turgidity also determine a loss of visual quality and texture with a decreased shelf-life (Cantwell et al., 1998; Løkke et al., 2012). Storing rocket at temperatures below 0°C can cause freezing of the leaves (Hall et al, 2013). Koukounaras et al. 2007 reported a shelf-life of 16 days when rocket leaves were stored at 0°C, while they observed a slight quality loss at 5°C, with a shelf-life of 13 days, and a rapid deterioration at 10°C with a shelf-life of 8 days. It is important to evaluate the product response, in terms of shelf-life of minimally processed leaves, in relation to different seasonal conditions

to select and improve the supply of quality produce. Hall et al. (2013) reported that the shelf-life of wall rocket leaves at 0°C was extended about 3 days compared to storage at 4°C and 6 days compared to storage at 7°C. Martínez-Sánchez et al. (2006b) found that during the storage of wild rocket leaves at 4°C, the visual quality can be maintained for 15 days after harvest using different sanitization methods.

For '*Eruca sativa*' at temperature of 0°C extended the shelf-life of 3 days compared to storage at 4°C and of 5 days compared to storage at 7°C. Other authors reported a shelf-life of 14 days for this species stored at 4°C (Martínez-Sánchez et al., 2006a; Nielsen et al., 2008).

1.5.3 Relative humidity

Relative humidity (RH) can influence water loss, decay development, incidence of some physiological disorders, and uniformity of fruit ripening. Condensation of moisture on the commodity (sweating) over long periods of time is probably more important in enchancing decay than is the RH of ambient air. The optimum relative humidity during storage of fresh non-fruit vegetables ranges between 95-98%, while for fruit and vegetables, except dry onions and pumpkins (70-75%) is about it is best kept at 90-95% (Kader, 2002a).

The physiological activity of plant tissue continues after harvest inducing wilting phenomena of tissue, a phenomenon primarily due to transpiration, i.e. the transfer of water contained within the plant tissue to the surrounding atmosphere. The dehydration depends on many factors including the temperature and relative

humidity of the storage room, the air movement and the packaging material. The weight loss is a natural consequence of the catabolism of horticultural products, catalysed by enzymes and accelerated by the handling and cutting. The decrease in weight may be attributed to respiration and other senescence-related metabolic processes during storage (Watada et al., 1999). Water loss is main cause of deterioration because it results not only in direct quantitative losses (loss of salable weigh), but also in losses in appearance (wilting and shrivelling), textural quality (softening, flaccidity, limpness, loss of crispness and juiciness) and nutritional quality. The commodity's dermal system (outer protective coverings) govern the regulation of water loss. The transpiration rate (evaporation of water from the plant tissues) is influenced by internal, or commodity, factors (morphological and anatomical characteristics, surface-to-volume ratio, surface injuries, and maturity stage) and by external, or environmental, factors (temperature, relative humidity, air movement, and atmospheric pressure) (Kader, 2002b).

Intact rocket leaves can be stored at 0°C with 95–100% RH (Cantwell, 2001). For rocket leaves water loss result in a dehydratation whic in turn affect firmnees lossed of the leaves (Cantwell et al., 1998; Løkke et al., 2012).

1.5.4 Gas composition

Modified atmosphere packaging (MAP) is a common technique used to extend the shelf-life period of fresh or minimally processed foods. This technique consists of modification of the air surrounding the product inside the package with another gas composition. MAP is usually used to store a wide range of horticultutal products;

the gas composition in the packaging is related to the type of product, packaging materials and storage temperature (Sandhya, 2010). During the storage, changes in the packaging occur due to the product respiration and the permeability of the film. For this reason, it is necessary to consider the permeability (for O_2 and CO_2) of the packaging film and assure that is adapted to the product respiration, in order to reach an equilibrium modified atmosphere in the package able to increase the shelf life of the product. Using low O_2 and/or high CO_2 concentrations it is possible to decrease the respiration rate, reduce the growth of postharvest pathogens, preserve the visual appearance, maintain nutritional quality, slow the browning process and the rate of deterioration during storage of fresh-cut products (Kader et al., 1989, Gorny, 2004)The primary gases used in modified atmosphere packaging are CO_2, O_2 and N_2. Carbon dioxide is a colourless gas and has a slight pungent odor when it is used at very high concentrations. CO_2 dissolves rapidly in water (1.57 g/ kg at 100 kPa, 20 °C) producing carbonic acid (H_2CO_3), that changes the acidity of reducing the pH. The high solubility of CO_2 can result in pack collapse causing the reduction of headspace volume. Oxygen is a colourless, odourless gas that is highly reactive and susceptible to combustion. It shows a low solubility in water (0.040 g/kg at 100 kPa, 20 °C). Oxygen can promote deterioration such as fat oxidation, browning reactions and pigment oxidation. Moreover, using low concentrations of oxygen can reduce the microorganism growth (O'Beirne et al, 2015) as bacteria and fungi. Nitrogen is a un-reactive gas with no odor, taste and color. Its density is lower than air, it has a low solubility in water (0.018 g/kg at 100 kPa, 20 °C) and in food constituents. Nitrogen alone does not support the growth of aerobic microbes,

while does not prevent the growth of anaerobic bacteria. CO_2 is considered a competitive inhibitor of polyphenol oxidase (PPO) enzyme involved in the tissues browning through the hydroxylation of monophenols to *o*-diphenols followed by oxidation to *o*-quinone; a reduction of PPO activity using CO_2 atmosphere compared with storage in air was observed in cold stored plant tissues (Lattanzio, 2003).

When the package is closed, no further control is possible and the gas composition will inevitably change due to produce metabolism and to film barrier properties (Sivertsvik et al. 2002). In addition appropriate gas composition for each type of product should be used, since tolerance to low oxygen and high CO_2 levels depend on the type of product. Outside of these limits physiological disorders with the production of undesirable metabolites may take place (Zagory et al.,1988; Soliva-Fortuny et al., 2001). One of major problems associated to storage in MAP is the accumulation of anaerobic metabolite as ethanol and acetaldehyde. Indeed, if the O_2 level in the MAP decreases below the fermentation threshold, anaerobic respiration is triggered leading to the production of off-flavours and stimulating the growth of some anaerobic psychrotrophic pathogens (Oms-Oliu et al., 2009). Similarly, injury will occur if CO_2 exceeds tolerable levels. Also, increases in ammonia were observed with high CO_2 atmospheres and were associated with darkening of tissues in some leafy green vegetables (Cantwell et al., 2009).

Postharvest quality management of rocket leaves can be a problem considering that leaves rapidly turn yellow, through the loss of chlorophyll, and are not suiTable for marketing. Leaf yellowing is caused by the chlorophyll degradation which is

enhanced by the presence of ethylene. Controlled atmosphere (CA) with elevated CO_2 level (5–8%) can decrease the chlorophyll degradation rate (Kenisgsbuch et al. 2014; Watada et al., 1996; Kader, 1995; Kader, 1986a; Yamauchi et al., 1993). Using controlled atmosphere with 5 kPa O_2 +10 kPa CO_2 (CA) improve the visual quality of wild rocket leaves stored at 4°C with a shelf life of 14 days after harvest (Martínez-Sánchez et al., 2006a). Retention of a dark-green color, with no yellowing or spots on the leaves, and texture are important for the perception of freshness by consumers and influences their choice, packaging of green leafy vegetables delays senescence and yellowing (Page et al., 2001; Toivonen et al., 2008) but anaerobic respiration can settle and induce loss of tissue integrity and development of an olive-brown color (Løkke et al., 2012). A correct management of gas composition could preserve the typical aroma of rocket, due to sulphurous compounds, with no development off-odors (Løkke et al., 2012). The same authors reported that using low O_2 concentration to store packed wild rocket leaves induces the loss of magnesium in the chlorophyll molecules and the dark-green color turn olive-brown (Toivonen et al., 2008). They found the using a film with high permeability to the oxygen (high oxygen transmission rate, OTR) preserved the "freshness" of produce, corresponded to retention of visual quality, color and odor, better than the storage with a low OTR film.

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Part two - Experimental

1.1 General objectives

This study is a part of the project "QUAFETY- Comprehensive Approach to Enhance Quality and Safety of Ready to Eat Fresh Products" that had the purpose to improve quality and safety of fresh-cut fruit and vegetables, including rocket salad (*Diplotaxis tenuifolia* L.) representing one of the most popular leafy vegetable on the market.

The shelf-life of ready-to-eat rocket is principally limited by the storage conditions and processing operations, which may accelerate the degradation process, inducing wilting, discoloration, loss of nutritional properties and sensorial attributes as aroma and flavor.

General aim of this thesis was to investigate the effect of several factors on the shelf-life of ready-to-eat rocket leaves in order to optimize the storage conditions through a correct management of the temperature and the gas composition inside the packaging. This objective was reached by steps which were related to the completion of partial objectives.

First objective of this work was to investigate the degradation kinetics of the major quality attributes of fresh rocket as a function of the temperature to better define the shelf-life and optimize the product logistic chain. For this experiment samples were stored in air.

The second objective was to independently evaluate for oxygen and carbon dioxide the effect of the concentrations on physical, sensorial and nutritional attributes of fresh rocket in order to individuate the optimal gas composition for packaging. This objective could be reached supplying the different gas levels in controlled

atmosphere. With the aim of analyzing the development of off-odors and the fate of characteristic aromatic compounds, volatile profile was also monitored in stressful conditions as the presence of high levels of CO_2. The accumulation of CO_2 is an event very common when packaging in modified atmosphere is not well designed, leading to the loss of the aroma and the production of off-odors. This condition may lead to very low final quality resulting in product rejection by the consumer once the package is opened.

Third objective of this work was to evaluate the effect of the temperature on rocket leaves packaged in passive modified atmosphere, where gas composition is in turn affected by the temperature. Passive modified atmosphere packaging is, in fact, the system commercially used for this kind of product. Degradation kinetics of the quality attributes on fresh-cut rocket were modeled in isothermal and non isothermal conditions in order to improve the management of the logistic chain according to the product thermal history. Also in this case, aroma profile, as obtained after homogenization of the leaves, was monitored simulating flavor development during chewing.

Finally a last experiment was aimed to monitor the volatiles accumulated in the headspace of the packages without enzymatic reactions induced by leave homogenization, to evaluate the effect of gas composition and temperature on the compounds responsible of the perceived odor and off-odors after opening of the package.

2.1 MODELLING SENSORIAL, PHYSICAL AND NUTRITIONAL CHANGES TO DEFINE QUALITY OF FRESH ROCKET (Diplotaxis tenuifolia)

2.1.1 Abstract

The aim of this work was to investigate the degradation kinetics of the major quality attributes in order to better define shelf-life of fresh rocket. Rocket leaves were stored in a humidified flow of air for 9 days in air at 5 °C, 15°C and 20°C. Sensorial, physical and chemical attributes such as appearance score, color, firmness, vitamin C, antioxidant activity and phenolic content were monitored during the storage. Attributes which showed significant changes over time were used to test conventional kinetic models of first order and Weibullian models. Weibull model showed an high ability to fit experimental data with high correlation coefficient (r) which ranged from 0.95 and 0.99 for all considered qualitative parameters while kinetic model of the first order always gave lower correlations. Appearance score, followed by ascorbic acid content were the parameters whish showed the highest rate of degradation and therefore can be used also for the prediction of the shelf-life. This information should be used to optimize the logistic chain with the aim to increase the quality of fresh rocket delivered to the final consumer.

2.1.2 Introduction

In the last 20 years the interest of the consumers on of fresh-cut fruits and vegetables increased exponentially because of their convenience as ready-to-eat

products and of the health benefits associated with their consumption (Martin et al., 2002; Oms-Oliu et al., 2009; Sothornvit et al., 2009). It is known that fresh-cut commodities enhance the intake of several nutrients present in horticultural products by facilitating their consumption. Main nutritional compounds of these products are minerals, vitamin C, vitamin A and thiamin as well as chemical compounds with functional properties such as fiber, carotenoids, flavonoids and antioxidants, which have benefits of human health (Gil et al., 2006). A diet rich in fresh-cut products may be useful to prevent cancer and cardiovascular diseases (Ames, 1983). Among leafy vegetables, the production of ready-to-use rocket arouses a great interest for its appreciated sensorial attributes such as pungent taste and bitter flavor. The shelf-life of ready-to-eat rocket leaves is principally limited by the storage conditions and processing operations, which may accelerate the degradation of the leaves, inducing wilting, discoloration, loss of nutritional properties, aroma and flavor (Koukounaras et al., 2009). Among the external variables affecting quality, temperature is a key factor for the overall degradation of fresh rocket through the control of all reaction rates, therefore may be useful to investigate the effect of temperature on fresh rocket attributes in order to improve the management of temperature for this product. The storage temperature is the principal factor to maintain the quality of rocket leaves, prolong their shelf-life (Kader, 2002; Koukounaras et al., 2007; Watada et al., 1996) and preserving visual appearance. Intact rocket leaves can be stored at 0°C with 95-100% RH (Cantwell, 2001); mechanical damages, or morphological defects influence negatively the visual quality of the product; leaves dehydration and loss of turgidity also determine

a loss of visual quality and texture with a decreased shelf-life (Cantwell et al., 1998; Løkke et al., 2012). Storing rocket at temperatures below 0°C can cause freezing of the leaves (Hall et al, 2013). Koukounaras et al. (2007) reported a shelf-life of 16 days when rocket leaves were stored at 0°C, while they observed a slight quality loss at 5 °C, with a shelf-life of 13 days, and a rapid deterioration at 10°C with a shelf-life of 8 days. It is important to evaluate the product response, in terms of shelf-life of minimally processed leaves, in relation to different seasonal conditions to select and improve the supply of high quality produce. Hall et al. (2013) reported that the shelf-life of wall rocket leaves at 0°C was extended about 3 days compared to storage at 4 °C and 6 days compared to storage at 7°C. Chlorophyll degradation has been reported to be the most critical postharvest alteration in rocket leaves resulting in yellowing; low temperatures can reduce the metabolic activity retarding the chlorophyll degradation. On these basis the modeling of the changes of visual appearance, physical attributes and the nutritional compounds is one of the most interesting research challenge which will help to better define the conditions for storability of fresh rocket. The design and optimization of the supply chain is in fact crucial for fresh produce (Dabbene et al., 2008; Jacxsens et al., 2010). An optimum logistic network should guarantee the high quality of fresh-cut fruits and vegetables also minimizing costs and maximizing sustainability indicators. Under these considerations the correct mathematical modelling of degradation reactions of a product is essential to obtain the best logistic network design. Quality degradation reactions are generally modeled using the conventional zero, first, or second order kinetics (Labuza, 1982; Tauokis et al., 1997; Zanoni et al., 2005; Nisha et al., 2005;

Giannakorou et al., 2003; Rekha Nisha et al., 2004; Rodrigo et al., 2007; Sothornvit et al., 2009); traditionally, the estimation of a kinetic constant is followed by use of the well-known Arrhenius equation to estimate rate constant at any temperature values to obtain a correct shelf-life prediction. Nevertheless, this methodology underwent to some criticisms (Corradini et al., 2004a; Corradini et al., 2004b; Corradini et al., 2006; Corradini et al., 2007) because sometimes the deterioration rate is not only a function of temperature but it depends also by time. Therefore other empirical or mechanistic models have been proposed (Peleg et al., 2002; Marabi et al., 2003; Corradini et al., 2007). Among these, Weibull model is the cumulative form of the Weibull distribution function that has also been used to describe the chemical and sensorial changes of fresh-cut produce (Iqbal et al., 2005; Oms-liu et al., 2009; Odriozola-Serrano et al., 2009). These models well fitted several food degradation reactions such as microbial growth (van Boekel, 2002; Corradini et al., 2004a; Corradini et al., 2007), antioxidant changes (Oms-Oliu et al., 2009), vitamin C degradation in frozen spinaches or green peas (Corradini et al., 2007), riboflavin degradation during thermal treatments (Corradini et al., 2006), browning of orange juice (Manso et al., 2001). The objective of the present work was to study the kinetic degradation of the most important physical and chemical changes of fresh rocket by comparing the conventional first order kinetics with Weibull models and to estimate the shelf-life of this product.

2.1.3 Materials and methods

2.1.3.1 Raw material and experiment setup

Fresh rocket leaves, *Diplotaxis tenuifolia,* were harvested in Salento (Apulia, Italy), washed in a free chlorine solution (0.01% v/v) before being drained, portioned in about 500g for sample and stored at 5°C, 15°C and 20°C in plastic containers (10 L) connected to a continuous and humidified flow of air in cold room respectively at 0°C, 5°C and 20°C. All quality rocket attributes were analyzed at 0, 1, 2, 5, 7 and 9 days of storage and analyzed on three replicates for temperature in each sampling day.

2.1.3.2 Physical and sensorial attributes

The "appearance" was scored by a group of panelist using a scale from 5 to 1 scale, where 5 = Excellent (fresh and turgid appearance, bright and uniform green colour), 4 = Good (slight loss of turgidity and fresh appearance), 3 = Fair (noticeable loss of turgidity and possible slight loss of green colour), 2 = Poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = Very Poor (severe yellowing of leafy blades and wilting, possible appearance of decay). Generally score 3 was considered as the limit of marketability, with the exception of firmness where samples too firm may not be accepted by the consumers, and 2 as the limit of not edibility.

Color was measured using a Spectral scanner (DV SRL, Italia), equipped with a Spectral Imaging spectrometer V10 type (400-1000nm, 25μm slit, resolution 5nm). One scansion per replicate was acquired, at speed of 3 mm/s in a dark room with a

stabilized halogen light source (150W). On the stored hyperspectral images a region of interest was selected for each cube (a square of 1 cm^2). The instrument software allowed to automatically measure the mean value of L*, a*, b* of the selected region in the CIE L*a*b* scale, elaborating the reflectance value to each pixel.

Firmness was measured as the rupture force (N) of a cube of 1 cm of side, pressed between 2 plates applying an increasing load at a speed of 30 $mm*min^{-1}$ with an Instron Universal Testing Machine (model 3340).

2.1.3.3 Chemical analysis

Total phenolic content and antioxidant activity were determined according to Singleton et al. (1965) and Brand-Williams et al. (1995) with minor modifications. Five grams of leafy tissues were homogenized in 2mM sodium fluoride methanol:water solution (80:20) for 1 min and centrifuged at 5 °C and 12,000×*g* for 5 min. The total phenol content was expressed as mg of gallic acid equivalent (GAE) 100^{-1} g fresh weight. The antioxidant activity was reported as mg Trolox equivalent antioxidant activity (TEAC) 100 g^{-1} FW. Reading were made at 725 nm, against a blank after 2 h standing for phenolic content and rat 515 nm after 24 h standing for antioxidant activity using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). Vitamin C were determined using five grams of fresh rocket tissue, which was homogenized with 10 mL of MeOH/H_2O (5:95) plus citric acid (21 g L−1) with EDTA (0.5 g L^{-1}). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined

as described by Zapata et al. (1992), with some modifications. The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20µl were analyzed with an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 v/v) containing 5mM cetrimide and 50mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. AA and DHA contents were expressed as mg of ascorbic or dehydroascorbic acid per kg of fresh weight (mg kg^{-1}).

2.1.3.4 Mathematical modeling

1. *Zero and First kinetic model*

Zero and first order kinetics, traditionally used to describes degradation reactions in foods, may be generally written as (Giannakourou et al., 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005)

$$\frac{dC_t}{dt} = -kC^m \qquad \text{(Eq.1)}$$

where $C_{(t)}$ is the concentration of the quality index at the time *t*, k is the rate constant and *m* is the kinetic order of the equation. The equation may be integrated easily obtaining the well-known decay functions. In particular zero order kinetic

model (m = 0) is written as $C_{(t)}=C_0$-kt whereas first kinetic order (m = 1) is $C_{(t)}=C_0e^{-kt}$.

2. *Weibull model*

According to Corradini et al. (2004), the degradation reactions can be analyzed using the cumulative form of the Weibull distribution:

$$F(t) = \exp(-bt^n) \qquad \text{Eq .1}$$

where $F(t)$ is the fraction of the molecules that retain their activity after time t, and b and n are model constants. Several physical, chemical and sensorial quality attributes were evaluated based on the fraction molecules that retain their "intact" form, which indicates their initial quality. For example, according to Corradini et al. (2006) and Jiang et al. (2012), we could consider the ascorbic and dehydroascorbic acids the "intact" and "failure" molecules, respectively. Generally, this approach could be applied to all analyzed quality attributes. Furthermore, $F(t)$ could be expressed as $C(t)/C_0$, where $C(t)$ and C_0 are the amounts of "intact" molecules at times t and at t = 0, respectively, thus the degradation reactions may be reported as follow:

$$C(t)/C_0 = \exp[-b(T)t^{n(T)}] \quad \text{Eq. 2}$$

where $b(T)$ and $n(T)$ are temperature-dependent coefficients.

In Eq. 2, $n(T)$ is the "shape factor", while the reciprocal of $b(T)$ is the "location factor".

The experimental data for each quality parameter were fitted using Eq. 2, and the estimated $b(T)$ and $n(T)$ for each storage temperature were fitted in Eq. 3 to estimate k and Tc. Curve fitting was obtained using Curve Fitting Toolbox of Matlab ver. 6.5

(MathWorks Inc, USA, 2002). The goodness of fitting was evaluated by the correlation coefficient (r), the sum of square error (SSE) and the root mean square error (RMSE). Moreover, the kinetic parameters were compared by the confidence interval calculated at the 95% of probability.

2.1.3.5 Statistical analysis

All data represent the mean of three replicates for each treatment and storage day. Data were subjected to analysis of variance for time of storage; significant differences among storage time were evaluated by Tukey's honest significance difference test ($p<0.05$) using Statgraphics Centurion XVI software.

2.1.4 Results and discussion

An ANOVA analysis was performed (data not shown) with the aim to evaluate if significant variation of the quality attributes occurred in relation to the storage time. Appearance, Chroma, firmness, vitamin C and phenols showed a similar trend characterized by a reduction over time. These attributes were fitted by first order kinetic model as well as by the Weibull model (eq. 2) and the results are reported in Table 1, with the exception of phenols at 20°C which could not be fitted due to the lacking of variation over time. In addition also antioxidant activity could not be fitted due to is trend enough stable over time (data not shown). Weibull model showed an high ability to fit experimental data with high correlation coefficient (r) which ranged from 0.95 and 0.99 for all considered qualitative parameters. Comparing the goodness of the fitting parameters between the models, Weibull

models showed correlation coefficients always greater (and in one case equal) than those obtained by conventional kinetic model of the first order (Table 1). Conventional models gave better results on Appearance and Chroma than for firmness, ascorbic acid and total phenolics. In fact, while r^* values ranged from 0.95-0.98 and from 0.897-0.95 for appearance and Chroma, respectively, much lower values were obtained for remaing parameters, being even lower than 0.6. According to several authors (Manso et al., 2001; Rekha Nisha et al., 2004, Rodrigo et al. 2007, Sothornvit et al., 2009; Oms-Oliu et al., 2009; Odriozola-Serrano et al., 2009) these results confirm that the conventional kinetics have a low flexibility to obtain a good interpolation in different conditions. On the other side, Oms-Oliu et al. (2009) obtained correlation coefficient always greater than 0.976 and and RMSE values minor of 1.002, fitting the changes of vitamin C content of fresh-cut melon samples stored between 5 and 20°C with the Weibull model. The same authors showed a correlation coefficient always higher than 0.986 when the total phenolic content as a function of time was fitted. Always on cut melons, Amodio et al. (2012) indicated that first was the apparent order of the quality change regarding L*, a*, b*, appearance score, fructose content, titraTable acidity, vitamin C, and phenol contents, and in another work, changes in appearance score, translucency, aroma, firmness and vitamin C of fres-cut melon have been described using of the Weibullian model (Amodio et al., 2013)

From the results reported in Table 1, it is clear that in the case of appearance score and Chroma value, experimental data could be well interpolated by both considered models, even if Weibull model was more accurate than conventional first kinetic

models, while firmness, vitamin C and phenols were best fitted using Weibull model. Table 1 also shows the estimated parameter values for location *b(T)* and shape *n(T)* factors obtained for the fitting with a Weibull model for each quality attribute and temperature (5, 15 and 20°C); the confidence intervals allowed to estimate the accuracy of the model in the interpolation of the qualitative d attributes of rocket leaves as a function of the temperature. Figure 1 shows the evolution of appearance score and the fit obtained by Weibull model. Panelists attributed an appearance score at the initial time of 4.93±0.09 corresponding to fresh and turgid appearance, bright and uniform green color (data not shown). Starting from the first day of storage, significant changes in the evaluation was detected between samples at 5, 15 and 20°C and the degradation rate substantially increased depending by temperature. The estimated scale factor *b* are 0.117, 0.126 and 0.278 for rocket stored respectively at 5, 15 and 20°C, while the shape factor are 2.08, 1.38 and 1.26 respectively for rocket at 5, 15 and 20°C, suggesting that differences of reaction rates between 0 and 15 °C are minimum, while at 20°C the 'b' factors is rapidly increasing being about two time higher than at 15°C .

For Chroma, the estimate *b* values are $1*10^{-6}$, 0.722 and 0.0106 for storage at 5, 15 and 20°C, while *n* values were 6.82, 1.88 and 5.92, for storage at 5, 15 and 20°C, denoting as different shapes were obtained at the different temperatures, and that degradation rate was much slower at 5°C. The location factor 'b', in fact, at 5°C resulted in a range from 10.000 to 100.000 lower than at higher temperatures, even if direct comparison of b values can not be done when shape factor are different. As for firmness (Figure 2), according to the idea that *n* values describe different

curve concavity, the shape of the fitting curve is different for the temperature at 15°C and more similar for the temperature at 5 and 20°C . Van Boekel (2002) reported, in fact, that *n* values >1 confer a downward concavity to the degradation curve, whereas *n* values <1 lead to an upward concavity. The calculated *b* values were 0.126, 0.0656 and 0.211 respectively at 5, 15 and 20°C, whereas *n* values of 0.251, 1.175 and 0.242 are obtained (Table 1). Also in this case, the 'b' values are similar for curve obtained at the temperatures of 5 and 20°C, indicating as degradation rate doubled passing from 5 to 20°C.

Figure 3 describes the fitting of experimental data of ascorbic acid content; starting from an initial value of 51.80±20.91 mg/100g FW (data not shown) ascorbic acid rapidely decreased during the storage. As for model coefficients, *b* values were 0.00144, $1*10^{-6}$ and 0.0031 respectively at 5°, 15° and 20°C, while *n* value were 3.09, 8.43 and 4.46 at the same temperatures; therefore direct comparison among 'b' factors can not be done. By observing the figure, is clear that degradation of ascorbic acid is much shorter at 5 °C, arriving at less than 30% of its initial value after 9 days, whereas it was completely consumed after 5 days at 15°C and 7 at 20°C.

Phenol content data fitting are reported in Figure 4; the initial amount was 86.74±12.51 mg of gallic acid/100g FW, which remained quite stable at 5°C. In Table 1 are reported *b* values estimated at 5, 15°C, since variation at 20°C, could not be fit. Values of estimated 'b' were were 0.0553 and $2.5 *10^{-6}$ corresponding to different *n* values, of 0.32 and 5.34 at 5, and 15°C, respectively. Also in this case

the degradation curve shows different shapes for the 2 temperatures of 5° and 15°C, with an higher degradation at 15 °C, starting after 6 days of storage.

From these results is possible to observe as appearance score and ascorbic acid degraded much faster than other attributes, and can be therefore used as target attribute to calculate the shelf-life. Considering score 3 as the limit of marketability for appearance, corresponding to a value of 60% of the initial value, a shelf-life of 6.11, 2.73 and 1.65 could be calculated at 5, 15 and 20°C, respectively. For the ascorbic acid loss shelf-life may be calculated as the number of days needed to reach a content of 20 mg $100g^{-1}$, which corresponds to 50% of the recommended daily intake as suggested by the Australia and New Zealand Food Authority, (2001). By using this reference limit a shelf-life of 7,22, 4,89 and 3,31 was estimated at 5, 15 and 20°C, respectively.

2.1.5 Conclusion

Weibull model could be used to fit sensorial, physical and chemical changes of fresh rocket better than conventional first-order models. Moreover, due to the high regression of the curve fitting, an accurate prediction of shelf-life was obtained. Among different attributes, appearance score and ascorbic acid, showed the highest degradation rate, being potential indicators for shelf-life. This information should be used to optimize the logistic chain with the aim to increase the quality of fresh rocket delivered to the final consumer.

2.1.6 References

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Table 1. Weibull parameters of the main quality indexes of the fresh rocket leaves stored in air at 0°, 5° and 20°C.

	Temperature (°C)	$b\ (d^{-1})$	Confidence interval	n	Confidence interval	r	r*
Qualitative attributes	**Appearance score**						
	5	0.117	0.0029-0.026	2.08	1.45-2.70	0.99	0.98
	15	0.126	0.0266-0.279	1.38	0.65-2.12	0.97	0.95
	20	0.278	0.047-0.509	1.26	0.24-2.28	0.99	0.97
	Chroma value						
	5	1*10-6	10^{-7}-0.0028	6.82	0.25-13.17	0.989	0.897
	15	0.0722	0.0044-0.140	1.88	1.25-2.51	0.996	0.95
	20	0.0106	0.0105-0.0107	5.92	5.91-5.93	0.999	0.915
	Firmness						
	5	0.126	0.068-0.184	0.251	0.01-0.518	0.96	0.57
	15	0.0656	0.001-0.200	1.175	0.0025-2.35	0.95	0.95
	20	0.211	0.081-0.340	0.242	0.01-0.666	0.95	0.64
	Ascorbic acid						
	5	0.00144	0.001-0.014	3.09	0.001-6.23	0.95	0.814
	15	$1*10^{-6}$	10^{-7}-$5*10^{-6}$	8.43	1.25-9.52	0.99	0.854
	20	0.0031	0.001-0.0282	4.46	0.12-11.61	0.98	0.932
	Phenols content						
	5	0.0553	0.0203-0.0904	0.32	0.02-0.67	0.99	0.329
	15	$2.5*10^{-6}$	$2.4*10^{-6}$-$2.6*10^{-6}$	5.34	4.62-6.06	0.98	0.779
	20	-		-	-	-	

r* is the correlation coefficient obtained by fitting experimental data with a conventional first-order kinetics equation.

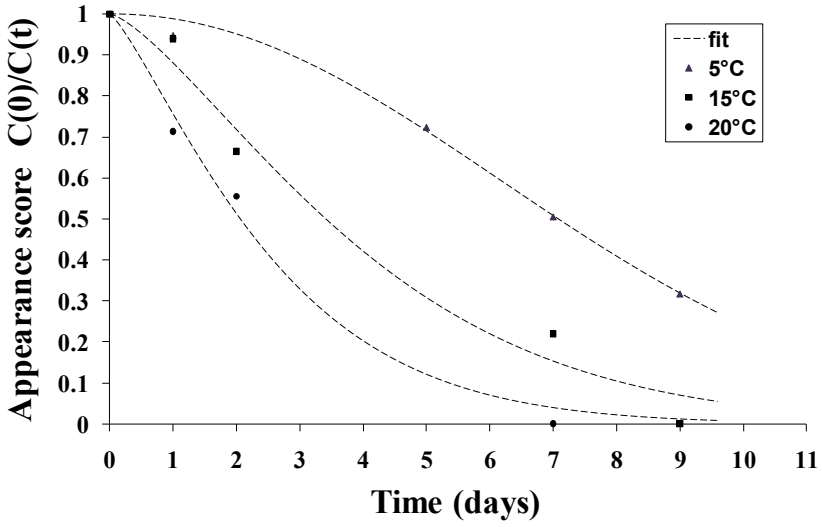

Figure1. Experimental value of appearance score of fresh rocket fitted by Weibull model at 5, 15 and 20°C.

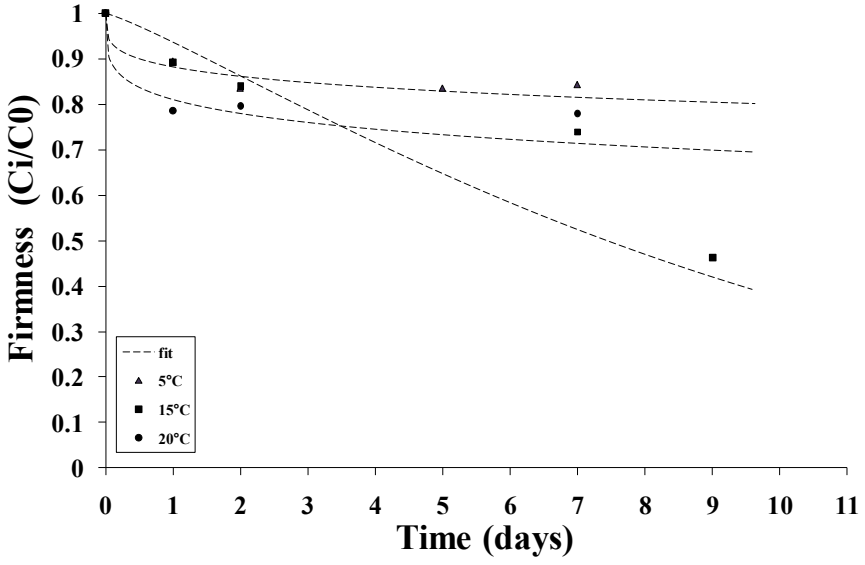

Figure 2. Experimental value of firmness values of fresh rocket fitted by Weibull model at 5, 15 and 20°C.

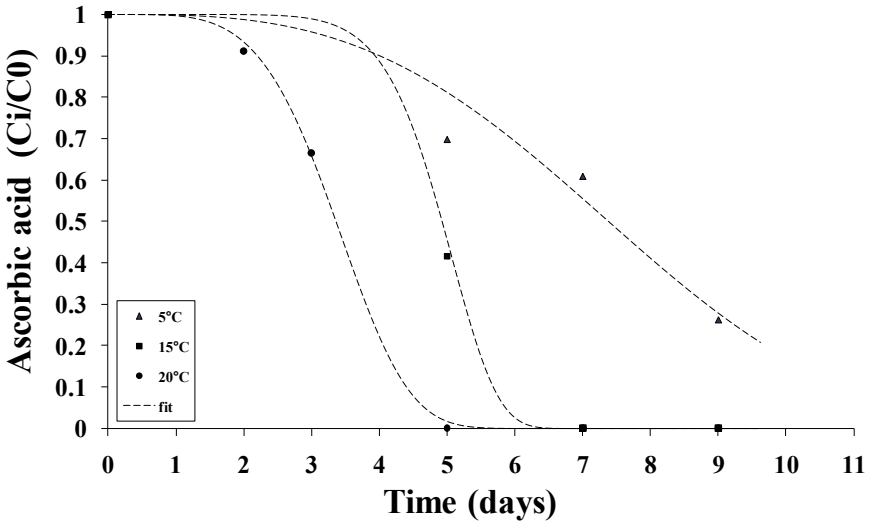

Figure 3. Experimental value of ascorbic acid content of fresh rocket fitted by Weibull model at 5, 15 and 20°C.

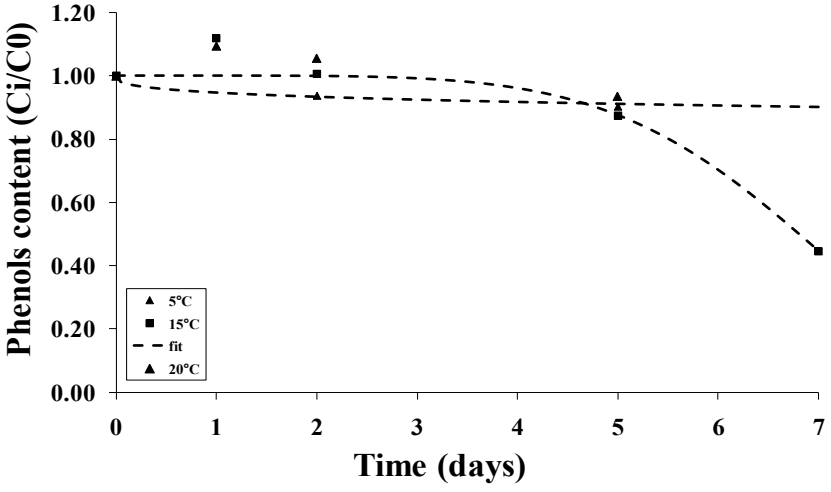

Figure 4. Experimental value of phenolic content of fresh rocket fitted by Weibull model at 5, 15 and 20°C.

3.1 EFFECT OF LOW O_2 ON THE STORABILITY OF ROCKET LEAVES (Diplotaxis tenuifolia)

3.1.1 Abstract

The aim of this work was to study the response of the rocket leaves to oxygen concentration to further optimize the packaging increasing theshelf-life. Rocket leaves were stored for 9 days at 5°C in a humidified flow of air as control, and in nitrogen with 0.5, 3 and 6 kPa O_2, respectively. Physical and chemical parameters as color, antioxidant activity, vitamin C, chlorophyll and phenolic contents were monitored at 0 time and after 1, 2, 3, 6, 9 days of storage at 5°C for all treatments. Marketability and sensorial evaluations were done to evaluate the acceptability of the product. No differences were observed in sensorial attributes among all treatments. Only appearance indicated that rocket showed higher quality when stored in air and 6 kPa O_2 than at lower oxygen concentrations. Color seemed to be best preserved using 0.5 kPa O_2, although this result is not supported by chlorophyll data. Differences were observed in vitamin C, total phenols content and antioxidant activity at the end of storage, but their values could be considered constant at the end of storage. In all treatments, no development of off-odors was observed. Physical and chemical parameters as color, antioxidant activity, vitamin C, chlorophyll and phenolic content were monitored at 0 time and after 1, 2, 3, 6, 9 days of storage at 5°C for all treatments. Marketability and sensorial evaluations were done to evaluate the acceptability of the product. No differences were observed in sensorial attributes among all treatments. Only appearance indicated

that rocket showed higher quality when stored in air and 6 kPa O_2 than at lower oxygen concentrations. Color seemed to be best preserved using 0.5 kPa O_2, although this result is not supported by chlorophyll data. Differences were observed in vitamin C, total phenols content and antioxidant activity at the end of storage, but their values could be considered constant at the end of storage. In all treatments, no development of off-odors was observed. Results indicated that storing fresh-cut rocket leaves in low O_2 concentration did not have an high impact on the quality of rocket leaves, but concentration as low as 0.5 kPa O_2 should be avoided.

3.1.2 Introduction

Wild rocket (*Diplotaxis tenuifolia*) is a cruciferous leafy vegetable popular in Europe often used raw alone or in a mixture of salad. The shelf-life of fresh-cut rocket is principally influenced by the storage conditions and processing operations, which accelerate the degradation of leaves resulting in wilting, discoloration, loss of nutritional properties, aroma and flavor (Koukounaras et al., 2009). Chlorophyll degradation, due to a yellowing process, was the most serious postharvest alteration in rocket leaves and can be retarded using low temperatures in order to reduce the metabolic activity. Rocket leaves can be stored at 0 °C for 16 days, increasing the temperature at 5 °C slight quality deterioration was observed with a shelf-life of 13 days (Koukounaras et al., 2007). In the market rocket leaves can be found unwashed or washed and packed in plastic trays generally wrapped in polypropylene (PP) film in order to create modified atmosphere packages (MAP). The gas concentration inside a MAP normally evolve to low O_2 and high CO_2

concentrations due to the product respiration. Low O_2 and high CO_2 are known to have beneficial effects on storability of fresh-cut product-by decreasing the respiration rate, the growth of postharvest pathogens and the deterioration rate (Kader et al., 1989). However, differences between beneficial and harmful atmosphere combinations may be small. In addition appropriate gas composition for each type of product should be used, since tolerance to low oxygen and high CO_2 levels depend on the type of product. Outside of these limits physiological disorders with the production of undesirable metabolites may take place (Zagory et al., 1988; Nielsen et al., 2008). The depletion of oxygen sufficient to ensure the control of enzymatic browning and to avoid anaerobic conditions, may results in the production of off-odors and off-flavor as in lettuce (O'Beirne et al. 2015). Atmosphere with low oxygen (5 kPa O_2 associated to different CO_2 levels) were reported to be beneficial for extending the shelf-life of rocket leaves (Martínez-Sánchez A. et al.; 2006), but any study verified the effect of oxygen alone on the quality of rocket leaves, and no information is available on the lowest O_2 concentration tolerated by this product. In this study we investigate the effect of different O_2 concentrations on quality attributes of fresh-cut rocket during the storage in order to find optimal conditions to preserve the quality and extend the shelf-life.

3.1.3 Materials and methods

3.1.3.1 Raw material and experiment setup

Fresh rocket leaves, *Diplotaxis tenuifolia,* were harvested in Salento (Apulia, Italy)

washed in a free chlorine solution (0.01% v/v) before being drained, portioned in about 500g for sample and stored at 5°C in plastic containers (10 L) connected to a continuous and humidified flow of air (control) and air enriched with 0.5, 3 and 6 kPa O_2. The quality of the rocket was observed for 9 days of storage and all parameters analyzed at 0, 1, 2, 3, 6, and 9 days.

3.1.3.2 Color analysis

Color of the rocket leaves was measured elaborating the images acquired with a Spectral scanner (DV SRL, Italia), equipped with a Spectral Imaging spectrometer V10 type (400-1000nm, 25µm slit, resolution 5nm). One scan of 10 leaves per sample was acquired with a speed of 3 mm min^{-1} in a dark room with a stabilized halogen light source (150W). On each rocket leaf, a region of interest (ROI) corresponding to the maximum inscribed rectangle was manually selected, allowing to calculate in the reflectance mode, the CIE L*, a*, b* scale colour parameters. Hue angle ($h° = \text{arctg}\frac{b^*}{a^*}$) and saturation ($\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$) values were calculated from primary L*, a* and b* readings.

3.1.3.3 Total phenolic content and antioxidant actvity evaluation

Total phenolic content and antioxidant activity were determined according to Singleton et al. (1965) and Brand-Williams et al. (1995) with minor modifications. Five grams of leafy tissues were homogenized in 2mM sodium fluoride methanol:water solution (80:20) for 1 min and centrifuged at 5 °C and 12,000×*g* for

5 min. The absorbance was read at 725 nm using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China) after 2 h standing for phenolic content, expressed as mg of gallic acid equivalent (GAE) 100^{-1} g fresh weight. For antioxidant activity the absorbance was red at 515 nm after 24 h standing The antioxidant activity was reported as mg Trolox equivalent antioxidant activity (TEAC) 100 g^{-1} FW. and).

3.1.3.4 Chlorophyll measurement

To determine the chlorophyll content, 0.5 grams of rocket leaves were immersed in 25 mL methanol for 24 hours at 20 °C in darkness. Chlorophyll quantification was performed spectrophotometrically at 653, 666 and 470 nm and the chlorophyll content was expressed as g per kg of fresh weight using the equation reported by Wellburn et al., (1994).

3.1.3.5 Sensorial analysis

The sensorial attributes of all samples were observed on day 0, 1, 2, 3, 6 and 9 of storage by a trained panel of five members. The appearance was scored using a scale from 5 to 1 scale, where 5 = Excellent (fresh and turgid appearance, bright and uniform green colour), 4 = Good (slight loss of turgidity and fresh appearance), 3 = Fair (noticeable loss of turgidity and possible slight loss of green colour), 2 = Poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = Very Poor (severe yellowing of leafy blades and wilting, possible appearance of decay). A score of 3 was considered as the limit of marketability. Off-odors and off-flavor

were scored on a 5 to 1 scale, where 1 = no off-odors/off-flavors, 2 = slightly off-odors/off-flavors, 3 = moderate off-odors/off-flavors (limit of marketability), 4 = strong off-odors/ off-flavors and 5 = very strong off-odors/off-flavors, sulfur compounds and rotten cabbage taste. A score of 3 was considered as the limit of marketability and a score of 1 as the limit of edibility. The same 5 points scale structure was used to evaluate the other attributes such as color (5 = typical green; 3= lost of green/beginning of yellowing; 1 = severe yellowing of leaves), firmness (5 = very crisp; 3 = slightly softening; 1 = severe softening), pungency (5 = very pungent; 3 = slightly pungent; 1 = no pungent), bitter (5 = very bitter; 3 = slightly bitter; 1 = no bitter), herbaceous (5 = very herbaceous; 3 = slightly herbaceous; 1 = no herbaceous), sweetness (5 = very sweet; 3 = slightly sweet; 1 = no sweet), and odor (3 = typical/strong, already perceptible on intact leaves; 2 = typical, perceptible on broken leaves ; 1 = slight, the odor perception was limited to rubbed and manipulated leaves).

3.1.3.6 Vitamin C analysis

Five grams of fresh rocket tissue were homogenized with 10 mL of MeOH/H_2O (5:95) plus citric acid (21 g L−1) with EDTA (0.5 g L^{-1}). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AsA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata et al. (1992), with some modifications. The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-

phenylenediamine dihydrochloride (OPDA). Samples of 20µl were analyzed with an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA).. The mobile phase was MeOH/H2O (5:95 v/v) containing 5mM cetrimide and 50mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. AA and DHA contents were expressed as mg of ascorbic or dehydroascorbic acid per kg of fresh weight (mg kg^{-1}).

3.1.3.7 Statistical analysis

All data represent the mean of three replicates for each treatment and storage day. Data were subjected to analysis of variance; significant differences among treatments and storage time were evaluated by Tukey's honest significance difference test ($p<0.05$) using Statgraphics Centurion XVI software.

3.1.4 Results and discussion

3.1.4.1 Effect of low oxygen treatments on sensorial evaluation

Sensorial attributes are very important for the consumer pint of view which expect a product to be fresh and without defects. Visual impact is the first attribute appreciable and influences the consumers choice, followed by odor retention at the opening of the packaging whereas taste is the last attribute to be evaluated. Sensorial analysis was carried out to assess changes on acceptability of rocket

leaves during the storage. Generally the gas composition did not show a high impact on the quality of stored rocket leaves. The perception of odor, off-odor, color, firmness, pungency, bitter, sweetness, greenless and off-flavor by the panellists were not affected by the oxygen level but only from the time of storage (Table 1). The appearance was influenced by treatments, temperature and their interaction (Table 1). In Figure 1 is reported the effect of low oxygen treatments on appearance and control in air for each storage time. Significant differences were found from the second days observing that appearance was slightly better preserved in samples stored in air than low oxygen treatment. Starting from day 3, the differences became wider, with rocket stored with 0.5 kPa O_2 showing the lowest values, being just below the marketability limit at the last day of storage, whereas samples stored in air and in 6% O_2 were judged still abov this limit. Off-odor evaluation showed a constant trend for rocket stored with 6 kPa O_2 at the end of storage (Figure 2), but not significant differences were observed between treatments compared with the samples in air (Table 1). It may be concluded that even the condition of 0.5% was higher that the anaerobic threshold. It is in fact knwon that storing vegetal tissues under an O_2 concentration required to ensure aerobic respiration, can induce anaerobic fermentation and development of off-flavor (Beaudry, 2000).

3.1.4.2 Effect of low oxygen treatments on color evaluation

Changes in color parameters during storage are reported in Table 1. Low oxygen treatments affected a* and b* values, Chroma and Hue angle, whereas time of

storage L* and b*, Chroma and Hue angle, with significant interaction except than for a*. The results could suggest a better retention of the green color in the samples stored with 0.5 kPa O_2 followed by 3 kPa O_2, air and 6 kPa O_2, as result of less variation compared to the initial values (data not shown) but differences were very small as can be also observed in Table 1 and not appreciable by the human eye. Evaluation of color by panelists in fact did not results in any difference in terms of color and yellowing among treatments. This is also supported by chlorophyll analysis which also did not show any effect of the treatment. Løkke et al. (2012) wrote that low O_2 concentration in packaged rocket leaves induces the loss of magnesium in the chlorophyll molecules causing the change of color from the typical dark-green to olive-brown (Toivonen et al., 2008). No darkening or yellowing processes could be seen in rocket leaves for the tested storage conditions.

3.1.4.3 Effect of low oxygen treatments on total phenolic content, vitamin C and antioxidant activity.

The treatments and storage time affected the antioxidant activity and vitamin C content, while changes observed in total phenol content depended only on treatment (Table 1). In this study, the antioxidant activity was 61.06±4.56 mg of trolox/100g FW at the zero time, samples stored in air and with 0.5 kPa O_2 showed a slight increase, respectively 25% and 19%, in the last days of storage, while samples stored with 3 and 6 kPa O_2 kept a similar content values during the storage (Figure 3). Total phenols content was 91.99±12.53 mg of gallic acid / 100g FW at zero time and showed a similar trend at the antioxidant activity during storage with a slightly

increase in samples stored in air and 0.5 kPa O_2. In the last day of storage total phenol content were 102.83±8.01, 98.75±8.93, 92.44±1.18, 89.30±4.71 mg of gallic acid/100g FW respectively in air, 0.5, 3 and 6 kPa O_2 treated rocket, but without significant differences (Figure 4).

Fruit and vegetables are a resource of vitamin C, which is biologically active compound and an essential nutraceutical involved in metabolic and physiological pathways in the human body; rocket leaves is one of green vegetables leafy to have an highest vitamin C content (Santos et al., 2012; Degl'Innocenti et al., 2007). In the vegetable tissues cells, it is available in two alternative forms, the ascorbic acid (AsA) and its oxidised form, L-dehydroascorbic acid (DHA); for this reason, vitamin C content is expressed as sum of the two forms. Rocket is a good source of vitamin C (Bennett et al., 2006). In this experiment, vitamin C content was 68.87±3.68 mg/100g FW at time zero and did not change much during storage. At the last day of storage slight higher values are found for rocket leaves stored in 6 and 3 kPa O_2 compared to the other treatments (Figure 5).

Santos et al. (2014) found no change in phenol compounds in fresh-cut rocket salad during the storage days and the increased antioxidant activity was associated to other compounds, including the vitamin C, which acted as oxidant agents; they suggested that vitamin C could be the main oxidant agents involved in the response against the oxidative stress. In this study the antioxidant activity trend (Figure 3) was more similar to that of phenolics content, but generally all of them showed little changes during storage with significant differences in the last days of the storage, in which the antioxidant activity was higher in samples stored in presence of air and

0.5kPa O_2 then samples stored in 3 kPa O_2 and 6 kPa O_2. Martínez-Sánchez A. et al. (2006) described changes in the vitamin C content during storage of rocket leaves in low oxygen conditions (5 kPa O_2 +5 kPa CO_2, 5 kPa O_2 +10 kPa C O_2), in air enriched with 10 kPa CO_2 compared to a control in air. They found an higher vitamin C content in rocket leaves stored in low O_2 than rocket leaves stored under the other conditions after 14 days of storage. In this study, the highest vitamin C content was detected by rocket stored under 0.5 kPa O_2 atmosphere and 6 kPa O_2 up to 6 days and as average value during storage in rocket stored with 0.5 O_2 (Table 1, Figure 5).

3.1.5 Conclusion

Three different concentrations of O_2 (0.5, 3 and 6 kPa) were tested to evaluate their potential beneficial effect on quality of fresh rocket leaves and at the same time to individuate if any negative effect could be observed in presence of low oxygen. Oxygen concentration did not have an high impact on quality of rocket leaves; no difference in sensorial attributes were appreciated by the panellists except that rocket stored under 0.5 kPa O_2 received a lower score at the end of the storage, compared to the other gas conditions, suggesting that O_2 concentrations below 0.5 kPa could decrease the shelf-life of rocket leaves. Antioxidant activity slighty increased during storage in 0.5 kPa O_2 and air, while total phenols and vitamin C were affected by treatments and storage time, but their trends cold be considered constant during storage suggesting that these oxygen concentrations did not induce metabolic stresses in rocket leaves if compared to rocket stored in air. In conclusion

even if the impact of the oxygen is not very critical, results of this experiment suggest to avoid concentration as low as 0.5 kPa.

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Table 1. Effect of low oxygen treatments, storage time and low oxygen treatment x time on the storage of fresh-cut rocket.

Attributes	air	0.5 kPa O	3 kPa O	6 kPa O	A: Treatment	B: Storage time	Interaction A x B
Appearance	4.03 a	3.82 c	3.96 b	4.04 a	****	****	****
Odor	1.61	1.65	1.67	1.65	ns	**	ns
Off-odor	1.14	1.14	1.12	1.04	ns	*	ns
Color	3.87	3.92	3.93	3.91	ns	****	ns
Firmness	3.57	3.56	3.51	3.62	ns	****	ns
Pungency	3.69	3.96	3.84	3.60	ns	ns	ns
Bitter	2.53	2.62	2.91	2.64	ns	ns	ns
Sweetness	1.16	1.12	1.12	1.12	ns	ns	ns
Herbaceous	2.32	2.2	2.405	2.52	ns	**	ns
Off-flavor	1.14	1.12	1.08	1.11	ns	ns	ns
L*	40.24	40.84	40.42	40.25	ns	**	*
a*	-15.67 ab	-15.76 b	-15.28 ab	-15.25 a	**	ns	ns
b*	18.04 a	18.33 a	17.09 b	17.04 b	****	****	****
Chroma	23.92 a	24.18 a	22.95 b	22.89 b	****	****	****
Hue angle	131.22 b	130.84 b	132.08 a	132.12 a	****	****	****
Chlorophyll (mg/100g)	86.76	97.61	93.00	98.27	ns	ns	ns
Total phenols (mg gallic acid/100g)	95.63 ab	98.65 a	91.31 bc	88.05 c	***	ns	ns
Antioxidant activity (mg Trolox/100g)	68.17 a	67.15 ab	61.51 bc	59.07 c	***	***	ns
Vitamin C (mg/100g)	70 ab	75.53 a	67.73 b	71.67 ab	****	***	ns

ns, not significant; * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ **** $P \leq 0.0001$

Mean values followed by different letter(s), are significantly different ($P < 0.05$) according to Tukey's honest significance difference test

(****) P ≤ 0.0001; (***)P ≤ 0.001; (**)P ≤ 0.01; (*)P ≤ 0.05; ns, not significant

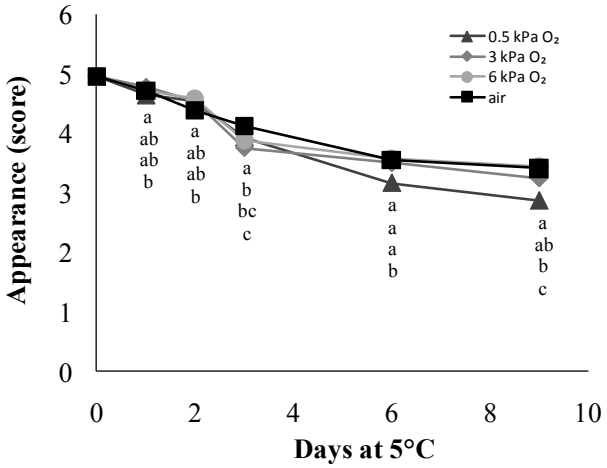

Figure 1. Effects of oxygen concentrations on the appearance score of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

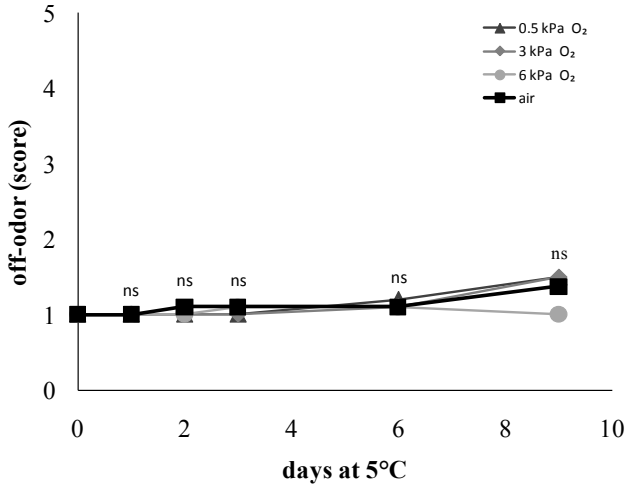

Figure 2. Effects of oxygen concentrations on off-odor score of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

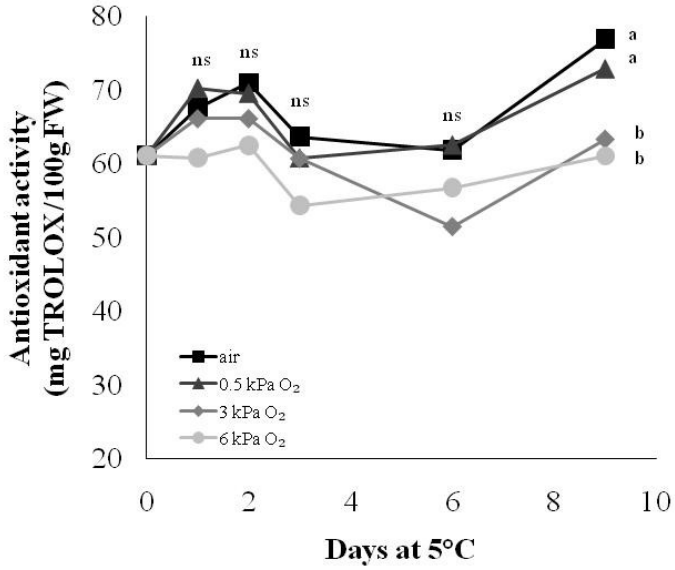

Figure 3. Effects of oxygen concentrations on the antioxidant activity of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

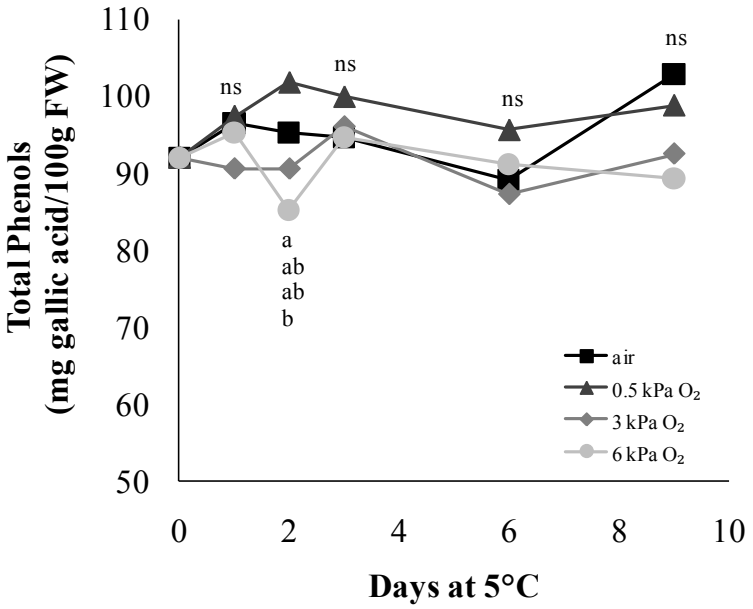

Figure 4. Effects of oxygen concentrations on total phenols of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

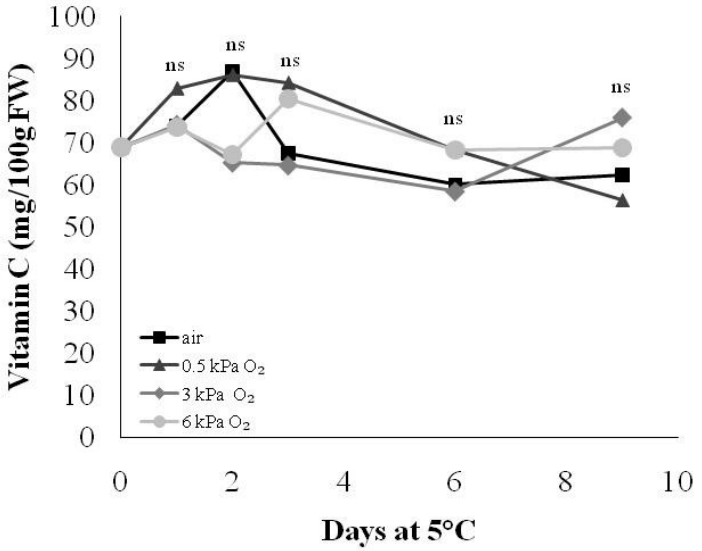

Figure 5. Effects of oxygen concentrations on vitamin C of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

4.1 EFFECT OF THE HIGH CO_2 ON THE STORABILITY OF ROCKET LEAVES (Diplotaxis tenuifolia)

4.1.1 Abstract

The aim of this study was to investigate the effect of high CO_2 concentrations on quality of fresh-cut rocket. Rocket leaves were stored for 10 days at 5°C in a humidified flow of air as control, and in air enriched with 5, 10 and 20 kPa CO_2, respectively. Physical, chemical and sensorial parameters as color, firmness, antioxidant activity, vitamin C, chlorophyll and phenolic contents were monitored initially and after 1, 2, 3, 6, 8 and 10 days of storage at 5°C for all treatments. Moreover volatile profile for rocket stored in air and in air enriched with 20 kPa CO_2 were also monitored in the headspace of the storage container. For most of the sensorial quality attributes, best results were observed for all treatments with high CO_2 concentration compared to control in air, even though the highest CO_2 concentration (20 kPa) induced the development of off-odors already from the third day of storage. In addition, at the end of the storage, also the rocket leaves stored in air showed the development of off-odors. Samples stored in CO_2-enriched atmospheres showed lower color changes than samples stored in air, with 10 kPa CO_2 resulting the best treatment for preserving rocket leaf appearance and this was confirmed by sensorial and colorimetric measures. Results indicated that storing fresh-cut rocket leaves in 10 kPa CO_2-enriched atmosphere was the most beneficial atmosphere to preserve quality compared to storage in air, and that higher CO_2 concentrations should be avoided.

4.1.2 Introduction

Wild rocket (*Diplotaxis tenuifolia*) is one of the most popular leafy vegetable in Europe, often eaten raw, alone or in a mixed salad. The shelf-life of ready-to-eat rocket leaves is principally limited by the storage conditions and fresh-cut processing operations, which may accelerate the degradation of the leaves, inducing wilting, discoloration, loss of nutritional properties, aroma and flavor (Koukounaras et al., 2009). Chlorophyll degradation has been reported to be the most critical postharvest alteration in rocket leaves resulting in yellowing; low temperatures can reduce the metabolic activity retarding the chlorophyll degradation. The rocket leaves can be stored successfully at 0 °C for 16 days, while at 5 °C a slight quality deterioration was observed and the shelf-life was reduced of 3 days. At 10 °C the rocket leaves deterioration was rapid with a shelf-life of 8 days (Koukounaras et al., 2007). Wild rocket is sold unwashed or washed and packed in plastic trays wrapped in polypropylene (PP) film to generate modified atmosphere packages (MAP) (Løkke et al., 2012), in which metabolic respiration determines the increase of the CO_2 levels inside the packaging reducing the O_2 levels. Generally, atmosphere with low O_2 and/or high CO_2 concentrations reduces the respiration rate, the growth of postharvest pathogens and deterioration rate during storage of fresh-cut products (Kader et al., 1989), but anaerobic conditions can occur with a development of off-odors. Nielsen et al. (2008) found an higher accumulation of off-odors in *Eruca sativa* stored in MAP and identified dimethyl sulphide and dimethyl disulphide as the volatile compounds causing a bad smell inside the packaging. An incorrect management of temperature also could induce the

production of off-odors. Moreover high levels of CO_2 in the package can also induce the development of physiological damages, when not tolerated by tissues, resulting in the necrosis of the tissues as reported for basil (Amodio et al. 2005) and for artichokes (la Zazzera et al., 2012). Regarding to the effect of the gas composition, Martìnez-Sànchez et al. (2006) found that the 5 kPa O_2+10 kPa CO_2-enriched atmosphere was effective in preserving a good appearance of the leaves if compared to the storage in air. Moreover the same authors reported that all the studied atmospheres (5 kPa O_2+5kPa CO_2, 5kPa O_2 +10 kPa CO_2 and air + 10 kPa CO_2) helped to maintain higher vitamin C contents than samples kept in air which also showed a more pronounced decrease in the total antioxidant capacity during storage. In the present study we further investigated the effect of different CO_2 concentrations even above the recommended value of 10 kPa CO_2 by Martìnez-Sànchez et al. (2006), on the quality attributes of fresh-cut rocket during the storage, also isolating the effect of the CO_2 by the synergistic effect of the low oxygen. The objective was to find optimal conditions for the packaging design to preserve the quality, and to study the impact of high CO_2 concentration (20 kPA) on volatiles and off-odors development.

4.1.3 Matirials and methods

4.1.3.1 Raw material and experiment setup

Fresh rocket leaves, *Diplotaxis tenuifolia,* harvested in a local field of the Apulia region (Italy) where washed in a free chlorine solution (0.01% v/v) before being

drained, portioned in about 300g for sample and stored at 5°C in plastic containers (10 L) connected to a continuous and humidified flow of air (control) and air enriched with 5, 10 and 20 kPa CO_2. The quality of the rocket leaves was monitored over a period of 10days and particularly at 0, 1, 2, 3, 6, 8 and 10 days.

4.1.3.2 Color analysis

Color of the rocket leaves was measured elaborating the images acquired with a Spectral scanner (DV SRL, Italia), equipped with a Spectral Imaging spectrometer V10 type (400-1000nm, 25µm slit, resolution 5nm). One scan of 10 pieces per sample was acquired with a speed of 3 mm min^{-1} in a dark room with a stabilized halogen light source (150W). On each rocket leaf, a region of interest (ROI) corresponding to the maximum inscribed rectangle was manually selected, allowing to calculate in the reflectance mode, the CIE L*, a*, b* scale colour parameters. Hue angle ($h°=\text{arctg}\frac{b^*}{a^*}$) and saturation ($\text{Chroma}=\sqrt{a^{*2}+b^{*2}}$) values were calculated from primary L*, a* and b* readings.

4.1.3.3 Textural analysis

The texture changes during storage were determined on five grams of leaves with an Instron Universal Testing Machine (model 3340), equipped with a Kramer cell. The leaves were placed in the Kramer shear cell equipped with 5 blades and extruded with the crosshead at a speed of 50 mm min^{-1}. Texture was measured as the maximum peak force and expressed in Newton (N).

4.1.3.4 Total phenolic content and antioxidant activity evaluation

Total phenolic content and antioxidant activity were determined according to Singleton et al. (1965) and Brand-Williams et al. (1995) with minor modifications. Five grams of leafy tissues were homogenized in 2mM sodium fluoride methanol:water solution (80:20) for 1 min and centrifuged at 5 °C and 12,000×*g* for 5 min. The total phenol content was expressed as mg of gallic acid equivalent (GAE) 100^{-1} g fresh weight. The antioxidant activity was reported as mg Trolox equivalent antioxidant activity (TEAC) 100 g^{-1} FW. Reading were made at 725 nm, against a blank after 2 h standing for phenolic content and at 515 nm after 24 h standing for antioxidant activity using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China).

4.1.3.5 Chlorophyll measurement

To determine the chlorophyll content, 0.5 grams of rocket leaves were immersed in 25 mL methanol for 24 hours at 20 °C in darkness. Chlorophyll quantification was performed spectrophotometrically at 653, 666 and 470 nm and the chlorophyll content was expressed as g per kg of fresh weight using the equation reported by Wellburn (1994).

4.1.3.6 Sensorial analysis

The sensorial attributes of all samples were scored by a trained panel of five members. The “appearance”, was scored using a scale from 5 to 1 scale, where 5 = Excellent (fresh and turgid appearance, bright and uniform green colour), 4 = Good

(slight loss of turgidity and fresh appearance), 3 = Fair (noticeable loss of turgidity and possible slight loss of green colour), 2 = Poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = Very Poor (severe yellowing of leafy blades and wilting, possible appearance of decay). A score of 3 was considered as the limit of marketability. “Off-odors” and “off-flavor” were scored on a 5 to 1 scale, where 1 = no off-odors/off-flavors, 2 = slightly off-odors/off-flavors, 3 = moderate off-odors/off-flavors (limit of marketability), 4 = strong off-odors/ off-flavors and 5 = very strong off-odors/off-flavors, sulfur compounds and rotten cabbage taste. A score of 3 was considered as the limit of marketability and a score of 1 as the limit of edibility. A The same 5 points scale structure was used to evaluate the other attributes such as color (5 = typical green; 3= lost of green/beginning of yellowing; 1 = severe yellowing of leaves), firmness (5 = very crisp; 3 = slightly softening; 1 = severe softening) and odor (3 = typical/strong, already perceptible on intact leaves; 2 = typical, perceptible on broken leaves ; 1 = slight, the odor perception was limited to rubbed and manipulated leaves)

4.1.3.7 Vitamin C analysis

Five grams of fresh rocket tissue were homogenized with 10 mL of MeOH/H_2O (5:95) plus citric acid (21 g L−1) with EDTA (0.5 g L^{-1}). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata et al. (1992), with some modifications. The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-

(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20 µl were analyzed with an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 v/v) containing 5mM cetrimide and 50mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. AA and DHA contents were expressed as mg of ascorbic or dehydroascorbic acid per kg of fresh weight (mg kg^{-1}).

4.1.3.8 Volatile analysis

For volatile evaluation additional container with 70 grams of product were stored in plastic jars (5 L) equipeed with an inlet and outlet plastic tubes . These container were flushed with a continue flow of air enriched with 20 kPa CO_2, maintaing the same flow rate proportion of the samples used for the other quality determinations. Volatile compounds were collected by solid-phase microextraction (SPME) using a 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) fiber and analysed by gas-chromatography (GC) coupled to mass spectrometry (MS). The fiber was exposed for 30 min to the exiting flow of gas of the plastic jars. Then, the fibre was withdrawn and introduced into the injector port of the GC for desorption a 250 °C for 4 min in the split injection mode (1:20).

An Agilent gas chromatograph model 6890 Series coupled to an Agilent 5975 C network mass selective detector was used. Analytes were separated on a DB-WAX capillary column (60 m x 250 μm x 0.25 μm) by applying the following temperature programme: 40 °C for 4 min, 40-200 °C at 3 °C/min, with a total run of 47.33 min. Transfer line temperature was 280 °C. Mass detector conditions were: electronic impact mode at 70 eV; source temperature 230 °C; scanning rate 2.88 scan/s; mass scanning range m/z 30-400. Carrier gas was helium at 1.0 mL/min. The identification of volatile compounds was achieved by comparing the mass spectra with the data system library (NIST 02, p>80). When available, the detected volatile compounds were compared with pure compounds. Three replicates were considered for the analysis in each sampling day.

4.1.3.9 Statistical analysis

All data represent the mean of three replicates for each treatment and storage day. Data were subjected to analysis of variance (one and two way anova); significant differences among treatments and storage time were evaluated by Tukey honest significance difference test ($p<0.05$) using Statgraphics Centurion XVI software.

4.1.4 Results and discussion

The effects of controlled atmosphere with different levels of CO_2 concentrations and storage time on quality parameters are reported in Table 1. Sensorial analysis were carried out to evaluate the acceptability of fresh wild rocket during storage. The overall appearance is the first appreciable attribute in a commercial fresh-cut

product, as the visual impact is the most important factor influencing the buying choice. Consumers purchase fresh cut rocket with an uniform color dark green and fresh appearance (Løkke et al., 2012) and discard yellow and rotten leaves, which are senescent and have an old appearance (Koukounaras et al., 2007). Fresh rocket stored with 20 and 10 kPa CO_2 were still marketable at 6 days of storage and edible at 10 days, whereas leaves stored in air and in air with 5 kPa CO_2 received a score lower than 3 (limit of marketability) already at 6 days (Figure 1). Particularly marketability of the leaves stored in air was limited by mould decay. After 10 days, samples stored with 5 and 10 kPa CO_2 received a score higher than samples stored in 20 KPa, and in air, which received the lowest evaluation. Moreover, air-stored rocket received at the end of the storage (10 days) a significant lower color score (Figure 2) than 10 and 20 kPa CO_2-stored samples, which indicate a more pronounced loss of the typical green color. The panelist observations are also supported by differences in chlorophyll (Figure 3) and color measures (Table 1). The chlorophyll content decreased during storage (Figure 3) with significant differences between the samples stored in CO_2 enriched atmosphere and in air; yellowing and loss of green color are considered the primary symptoms of the chlorophyll degradation (Brown et al., 1991; Heaton et al., 1996).

The color parameters were affected by the treatment, the storage time and their interaction (Table 1). The lightness, b* and Chroma values increased in the samples stored in air at the end of the storage, as consequence of the yellowing process (data not shown). Generally atmosphere with the highest concentration (20 kPa) helped to preserve chlorophyll and color (Table 1), even though the visual appearance after

10 days was judged lower compared to samples stored with CO_2 concentrations up to 10 kPa. These results confirmed what reported by Martínez-Sánchez A et al. (2006), even though these authors referred that most of the treatments were still marketable after 10 days. The lower shelf-life found in the present work may be attributed to some differences in the raw material, most likely due to the agricultural practices or the climate (Pareira et al., 2013). Another important quality attribute for consumer is the aroma which can be only evaluated once the packaging is opened. Despite a retention of a good visual appearance, panelists judged 20 kPa of CO_2-stored rocket no marketable already at 6 days of storage, due to the sensible development of off odors (Figure 4) (score higher than 4), which were described with "chemical" notes. At the end of the storage, air-stored samples were also judged not marketable, with notes of "cabbage", whereas the panellists felt moderate off-odors in the rocket stored in air with 5 and 10 kPa CO_2. Exposing the fibre to the headspace of the plastic container, in which 20 kPa CO_2-enriched atmosphere was flushed, allowed to sample the most abundant volatile compounds produced during the storage. Eight compounds were detected, included dimethyl sulphide, dimethyl disulphide, responsible for the perception of off-odors in rocket leaves (Nielsen et al, 2008), 2-butanone and 2-pentanone. In Table 2, chromatographic peak areas of volatile compounds found in rocket in the first day and in the last day of storage (day 10), for air and 20 kPa CO_2 treatments, are reported. Derbali et al. (1998) reported that broccoli seedling stored under anaerobic conditions produced dimethyl sulphide and dimethyl disulfide through the conversion of protein-derived cystein. However, dimethyl disulfide can also derive

by the oxidation of methanethiol (Lindsay et al, 1986) or by thermal degradation of sulforaphane in Brassicacea species (Jin et al., 1999). We reasonably suppose that all these processes can occur in rocket leaves giving a different contribution to the dimethyl disulfide development. Dimethyl sulphide and dimethyl disulfide confer note of sulphurous, cabbage and onion-like; the higher production in rocket stored in air then in rocket stored with 20 kPa CO_2 (Figure 7-8) could depend on the major rate of oxidation reactions yielding to dimethyl disulphide and dimethyl sulfide, while the presence of oxygen also in 20 kPa CO_2-stored rocket inhibited fermentative processes resulting in a low presence of these compounds. Moreover, it's not possible to exclude a bacteria contamination of the rocket leaves stored in air, compared to the leaves stored in high CO_2 and that the production of dimethyl sulfide and disulfide in air stored samples was due also to the presence of these microorganisms. Lonchamp et al. (2009) reported that the terpene D-Limonene could be considered as a marker for green color loss through the chlorophyll degradation in lettuce and could be an oxidation product of chlorophyll in the leafy green vegetables. In the present study, D-limonene content did not change during the storage and no differences were found comparing both treatments. We observed a slight production of ethanol during the storage, but no significant differences were detected between air and 20 kPa CO_2-stored rocket leaves; very often its detection was below the sensitivity limit likely because aerobic conditions were maintained also in CO_2-treated samples. In the last days of the storage, rocket leaves stored with 20 kPa CO_2 developed the highest quantity of 2-butanone (Figure 5) and 2-pentanone (Figure 6), described with solvent and nail polish odors, respectively. On

the other side, samples stored in air showed an increased production of dimethyl sulphide (Figure 7) and dimethyl disulfide (Figure 8), sulphide odors tipical of *Brassicacea* family described as cabbage and garlic-like. Despite the different chemical nature of off-odors developing during storage under these two gas conditions, the same level of intensity of off-odor was perceived by the panellist at the end of the storage (Figure 4).

4.1.4.1 Antioxidant compounds

The atmosphere composition and storage time affected the total phenol content; in particular way, total phenols were significantly higher in the samples stored in presence of CO_2 than in samples stored in air. The initial content was 73±5 (mg of gallic acid/100g FW) while the final content was slightly low for all treatments, including the control in air (Figure 9). There are many evidences in the literature about the effect of controlled atmosphere on the phenolic compounds of some fruit and vegetables. Gil et al. (1998) reported that the phenolic content in green lettuce tissues, in which aerobic conditions were maintained, significantly decreased during storage, whereas, on the contrary, it increased in MAP. Amodio et al (2014) developed a kinetic model to describe the changes in the phenolic content of fresh-cut produce during storage, the phenolic trend is characterized by significant increase during the first days of storage. Total phenols of the rocket samples stored under CO_2-enriched atmosphere showed similar trends with a slight increase in the first days of the storage, whereas samples in air showed an initial decrease which could be due to a much enhanced oxidation process. Rocket leaves is one of green

vegetables leafy with the highest vitamin C content (Santos et al., 2012; Degl'Innocenti et al., 2007). Vitamin C is the generic term to define the L-ascorbic acid (AsA), the biologically active form in the vegetable tissues. In the tissues cells, AsA is in part oxidised in L-dehydroascorbic acid (DHA), for this reason, vitamin C content is expressed as the sum of the two forms. AA is the predominant form in fruits and vegetables; prolonged storage, mechanical and thermal treatments are responsible for the oxidation of AA in DHA (Wechtersbach et al., 2011; Davey et al., 2000; Lee et al., 2000). It is known that both forms have a biological activity (Cabezas-Serrano et al., 2009, Deutsch, 2000). Nevertheless, the human intestinal cells can absorb and reduce DHA in AA, which is then injected in the blood (Wilson, 2002). The rocket vitamin C decreased during the storage without significant differences between storage treatments until the last two day of storage, when the rocket stored with 20 kPa CO_2 held a major content of vitamin C (Figure 10).

The antioxidant activity increased during the storage time up 60-70% (data not shown), but no significant differences were observed among treatments. Those results were in according to Santos et al. (2014) who reported that phenol compounds in the ready-to-eat rocket salad did not change during the storage days and that increased antioxidant activity could be associated to other compounds.

4.1.5 Conclusions

Three different concentrations of CO_2 (5, 10 and 20 kPa CO_2) in addition to the control in air were tested to understand their beneficial and negative effects on the

fresh-cut rocket storage. All treatments showed a similar trend until the 6 day of storage for the vitamin C, chlorophyll and phenolic content. The physiological stress induced using high CO_2 levels, was evident at the last days of storage when the product was considered not marketable by the panellists. The effects of high CO_2 were much evident in the sensorial analysis starting from the sixth day of storage. At this time, even if the appearance was better preserved for samples stored in presence of 20 kPa CO_2 than for rocket stored in air, a strong production of off-odors was perceived. This condition did not occur during the storage of rocket with 10 kPa CO_2. The off-odors perceived during the storage in air could be associated to dimethyl sulfide and dimethyl disulfide content, while in rocket stored in presence of 20 kPa CO_2 the major contribute to the perception of altered odor could depend by production of 2-butanone and 2-pentanone. These results indicated that storing fresh-cut rocket leaves in 10 kPa CO_2-enriched atmosphere was the most beneficial atmosphere to preserve quality compared to storage in air; higher concentrations should be avoided.

4.1.6 References

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Table 1. Effect of CO_2 concentration, time of storage and treatment x time of storage on quality attributes of fresh rocket leaves.

Attributes	air	5 kPa CO	10 kPa CO	20 kPa CO	A: Treatment	B: Storage time	Interaction A x B
Appearance	2.64 c	3.23 a	3.20 a	3.09 b	****	****	****
Color	3.21	3.4	3.59	3.76	*	****	ns
Off-odors	1.71 b	1.69 b	1.67 b	2.46 a	**	****	**
L	46.02 a	44.10 b	43.68 bc	42.89 c	****	****	****
a*	-17.48	-17.56	-17.65	-17.36	ns	****	****
b*	25.11 a	23.26 b	22.79 b	21.94 c	****	****	****
Chroma	30.68 a	29.18 b	28.86 b	28.01 c	****	****	ns
Hue angle	125.16 b	127.26 ab	127.92 a	127.24 ab	*	***	ns
Firmness (N)	180.31	174.93	174.56	172.8	ns	**	****
Total phenols (mg gallic acid/100g FW)	70.44	73	74.45	76.02	ns	**	*
Antioxidant activity (mg Trolox/100g FW)	58.75	59.55	59.43	58.2	ns	****	ns
Chlorophyll (mg/100g FW)	64.61 b	71.81 ab	73.93 ab	76.29 a	*	****	ns
Vitamin C (mg/100g FW)	25.22	25.77	27	24.77	ns	****	ns

ns, not significant; * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ **** $P \leq 0.0001$

Mean values followed by different letter(s), are significantly different ($P < 0.05$) according to Tukey's honest significance difference test

(****) P ≤ 0.0001; (***)P ≤ 0.001; (**)P ≤ 0.01; (*)P ≤ 0.05; ns, not significant

Table 2. Effect of 20 kPa CO_2 treatment on volatile composition of fresh rocket leaves at 0 and 10 days of storage (Values of peak area are divided by a factor of 10^6 ; different letters after values of peak area mean significant differences for ($P<0.05$). Odor descriptors using published data (Jirovetz et al. 2002 and references therein; Sigma-Aldrich, 2001)

volatile compound	rt	AIR (t0)	20 kPa CO_2 (t10)	AIR (t10)	odor descriptor
dimethyl sulfide	5.58	2.38±1.06	7.29±6.74 b	373.48±55.21 a	sulfurous
2-methyl-furan	7.57	nd	nd	nd	etereal, acetone
ethyl acetate	7.94	4.62±1.76	2.43±1.35	0.62±0.25	etherial, fruity, sweet, grape, rum-like
2-butanone	8.33	1.32±0.17	14.91±5.27 a	1.80±0.93 b	etherial, diffusive and slightly fruity with a camphoreous nuance
ethanol	9.50	3.50±2.07	nd	3.53±3.08	alcoholic, ethereal
2-pentanone	10.81	nd	7.69±6.29	nd	etherial, diffusive, sweet banana-like fermented woody nuance
methyl isocyanide	11.72	0.86±0.34	0.88±0.74	1.10±0.67	
thiophene	12.51	nd	nd	nd	alliaceous garlic
ethyl butanoate	13.14	2.15±0.84	1.14±0.54	0.29±0.15	sweet, fruity,
dimethyl disulfide	14.66	nd	16.33±22.64 b	214.39±50.35 a	sulfurous
D-limonene	20.39	5.26±2.95	6.01±4.36	6.36±2.91	citrus, sweet, lemon
ethyl hexanoate	22.18	1.97±1.44	1.21±0.17 a	0.54±0.15 b	sweet, fruity, pineapple, waxy, fatty

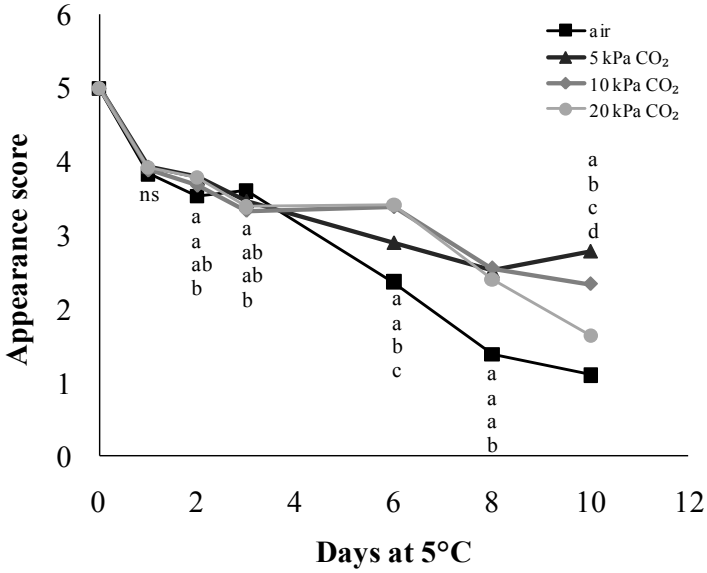

Figure 1. Effect of CO_2 concentration on appearance screof the fresh rocket leavesstored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

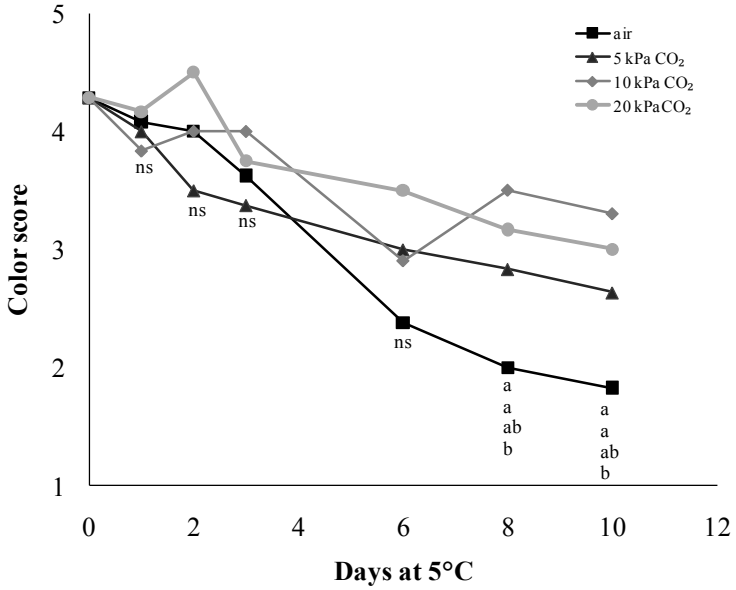

Figure 2. Effect of CO_2 concentration on color score of the fresh rocket leaves stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

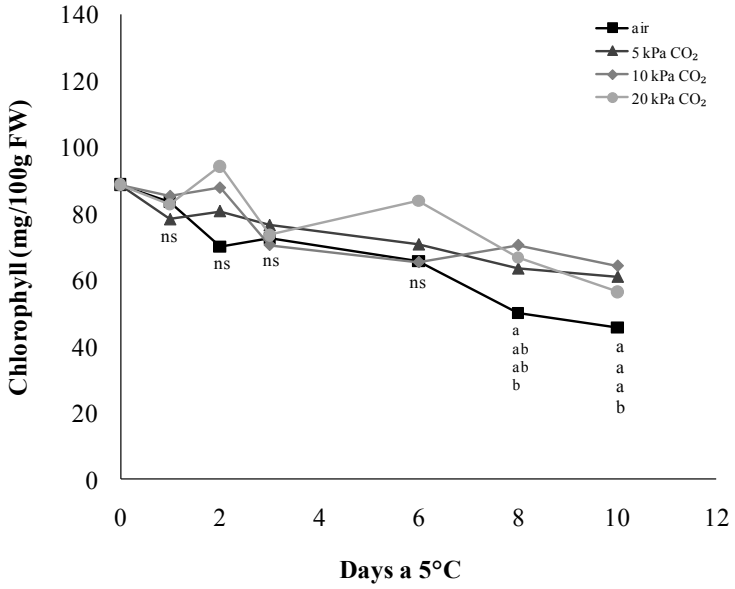

Figure 3. Effect of CO_2 concentration on chlorophyll content of fresh rocket leavesstored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

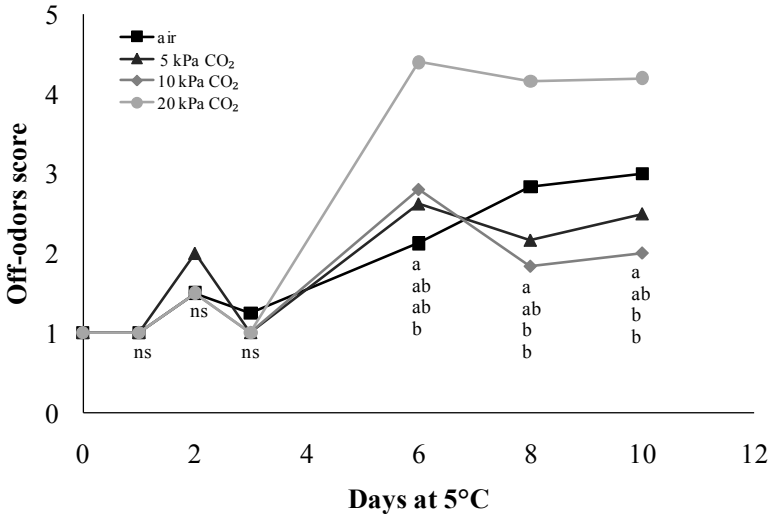

Figure 4. Effects of CO_2 concentration on the Off-odors evaluation of the fresh-cut rocket stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

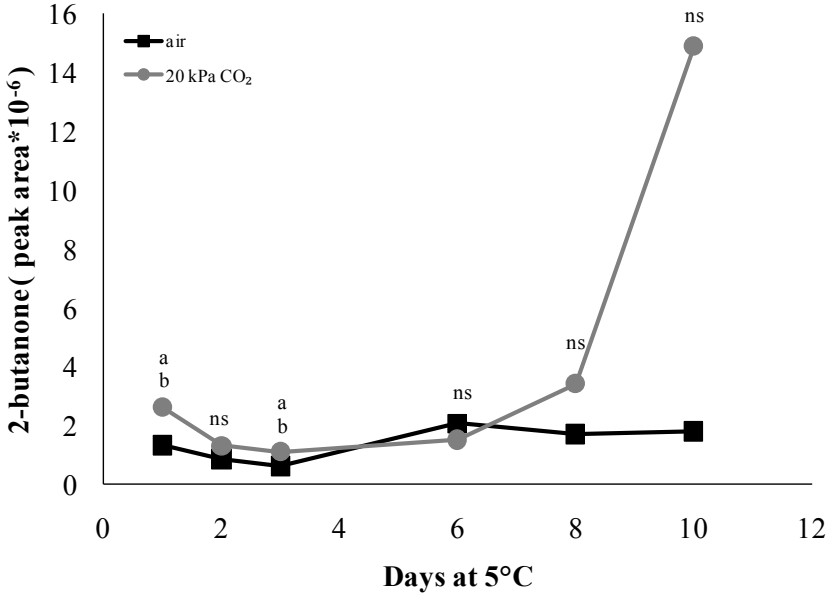

Figure 5. Development of 2-butanone on fresh rocket leaves stored at 5 °C in air and in air enriched with 20 kPa CO_2. Mean values of 3 replicates. Values of peak area are divided for a factor of 10^6; different letters after values of peak area mean significant differences for ($P<0.05$).

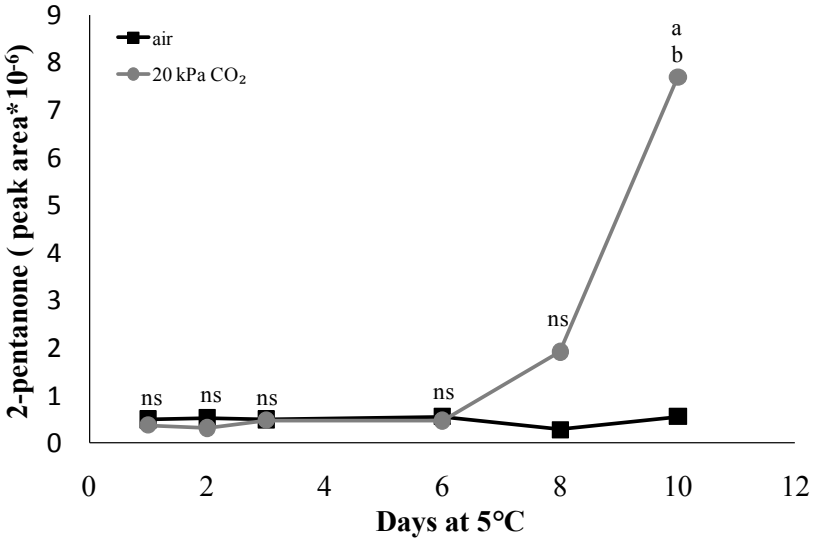

Figure 6. Development of 2-pentanone on on fresh rocket leaves stored at 5 °C in air and in air enriched with 20 kPa CO_2. Mean values of 3 replicates. Values of peak area are divided for a factor of 10^6; different letters after values of peak area mean significant differences for ($P<0.05$).

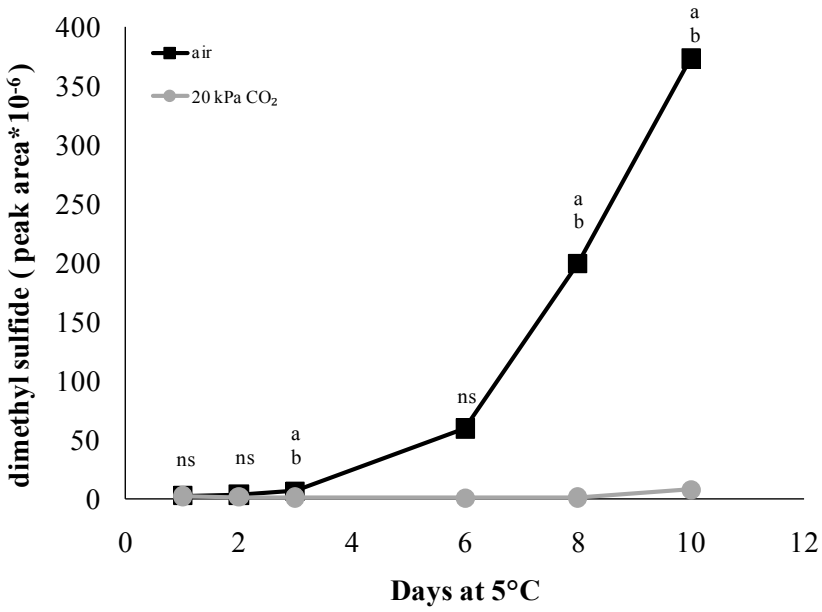

Figure 7. Development of dimethyl sulfide on fresh rocket leaves stored at 5 °C in air and in air enriched with 20 kPa CO_2. Mean values of 3 replicates. Values of peak area are divided for a factor of 10^6 ; different letters after values of peak area mean significant differences for ($P<0.05$).

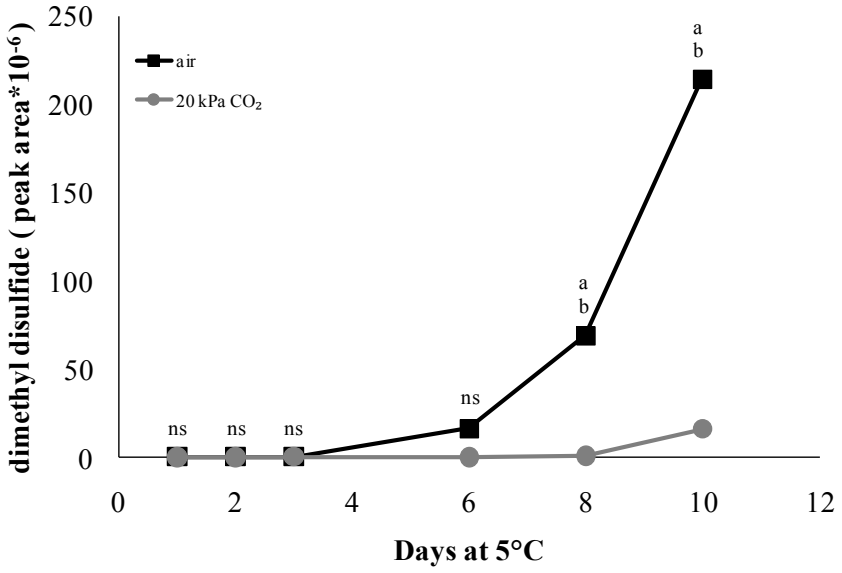

Figure 8. Development of dimethyl disulfide on on fresh rocket leaves stored at 5 °C in air and in air enriched with 20 kPa CO_2. Mean values of 3 replicates. Values of peak area are divided for a factor of 10^6 ; different letters after values of peak area mean significant differences for ($P<0.05$).

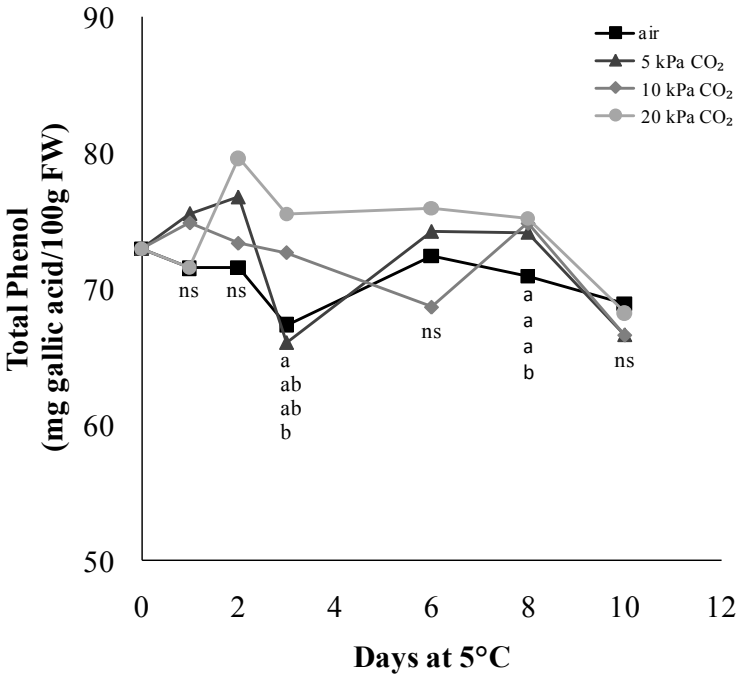

Figure 9. Effect of CO_2 concentration on total phenol content of fresh rocket leavesstored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

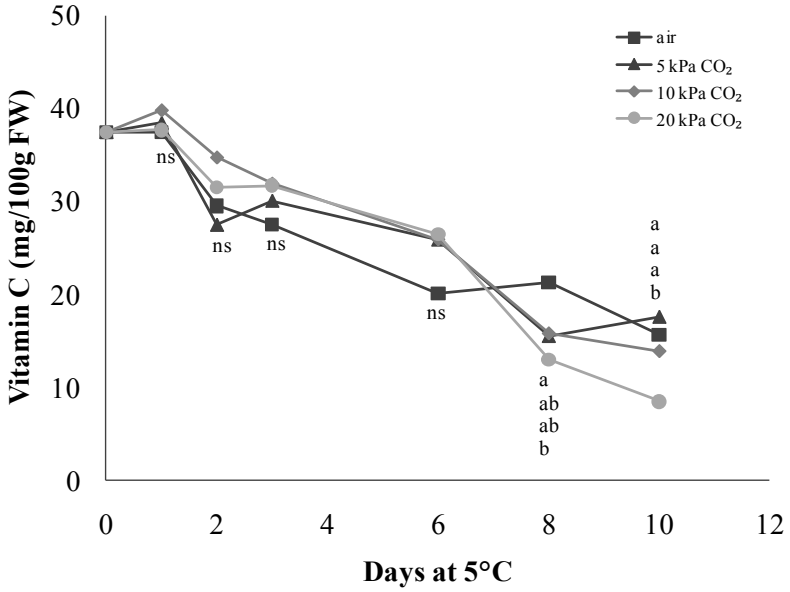

Figure 10. Effects of CO_2 concentration on the vitamin C content of the fresh-cut rocket stored at 5 °C. Mean values of 3 replicates. Different letters at different time of storage mean significant differences for ($P<0.05$).

5.1 A STUDY OF THE ESTIMATED SHELF LIFE OF FRESH ROCKET USING A NON-LINEAR MODEL

5.1.1 Abstract

The shelf life (SL) of fresh-cut rocket leaves stored at 0, 5 and 15°C was studied using a non-linear model. In particular, the cumulative form of the Weibull equation and a log-logistic model were, respectively, used to fit the experimental data over time and to study the temperature dependence of the degradation rates for several sensorial, chemical and physical attributes. The Weibullian model fit the experimental data, exhibiting correlation coefficients between 0.950 and 0.996; when using traditional first-order kinetics, this value ranged from 0.902 to 0.985. Additionally, a log-logistic model that could accurately describes the temperature dependence of the Weibull parameters also accounting for the thermal history of the product. Due to the different degradation patterns of the degradation curves, the definition of the shelf life significantly changed based on the quality attributes and the storage temperatures. In particular, when the samples were stored at 5°C, the appearance scores limited the shelf life, exhibiting values of 5.8 days; when non-isothermal conditions were considered, the ascorbic acid content became the critical factor due to its major sensitivity toward temperature abuse. Results may be useful for planning produce logistics with fully automated distribution steps and better managing stocks, according to thermal history and, possibly, the priorities of potential customers.

5.1.2 Introduction

Rocket (*Eruca Sativa* Mill.) is one of the most appreciated leafy vegetables in Europe, where it is traditionally used alone, in mixed salad, or as a popular pizza topping and is sometimes cooked with pasta. The most important quality attributes of this vegetable include its pungent taste and sharp, spicy and peppery flavor. The taste of rocket is associated with its glucosinolate content and the associated breakdown products; in addition, more than 20 volatile compounds are responsible for its characteristic flavor (Bennet et al., 2006; Nielsen, et al. 2008). Furthermore, rocket leaves have high contents of health-promoting phytochemicals, including carotenoids, vitamin C, flavonoids and glucosinolate (GLs) (Barillari et al., 2005; Bennet et al., 2002; Melchini et al., 2009). The leaves may be sold unwashed or as a ready-to-eat product, which is washed, dried and packed under a modified atmosphere (MAP) to obtain a shelf life of 10 days. During storage, rocket leaves quality rapidly degrades, particularly through yellowing caused by chlorophyll degradation, wilting, and the production of off-odors (Koukounaras et al., 2006; Siomos et al., 2007; Nielsen et al., 2008; Koukounaras et al., 2009). The perishability of rocket is attributed to the high respiration rate and the sensitivity of the leaves toward exogenous ethylene (Koukounaras et al., 2007). Using a MAP, in addition to low temperatures, is the most important tool for increasing the shelf life; these conditions decrease the oxygen concentration while increasing the carbon dioxide concentration, reducing the rates of the degradation reactions (Kader et al., 1989; Escalona et al., 2006; Kalio, 2008). Rocket should be stored at 0°C with 95-100% RH (Cantwell, 2001); however, the leaves are often stored between 5 and

10°C (Watada et al., 1996). Moreover, improper packaging can lead to anaerobic conditions that induce the production of off-odors, which can be perceived only after opening the packaging (Løkke et al.,2012). Consequently, understanding the kinetic degradation of rocket leaves under different storage conditions is essential when designing storage condition and estimating the shelf life of this product. In general, conventional zero-, first- and second-order kinetics have been used to study the degradation reactions in fresh and processed horticultural products (Giannakourou et al., 2003; Nisha et al., 2005; Pedro et al. , 2006; Sothornvit et al., 2009; Odriozola-Serrano et al. 2009). Once the degradation rate (*k*) values are estimated at different storage temperatures (from accelerated storage experiments), the Arrhenius model has been traditionally used to describe the temperature dependence through the activation energy (Ea) of the reaction (Giannakourou et al. 2003; Nisha et al., 2005; Nisha Rekha et al., 2004; Sothornvit et al., 2009). However, Corradini et al. (2006 and 2007) stated that this approach could result in significant discrepancies between the estimated shelf life and the experimental results, particularly under non-isothermal storage conditions. Using traditional zero-, first- and second-order kinetics often significantly reduces the goodness of fit (Odriozola-Serrano et al., 2009; Sothornvit et al., 2009) due to their low flexibility, which increases the uncertainty of the predicted shelf life. Moreover, one of the most important drawbacks is that the Arrhenius equation is derived while assuming that all of the degradation reactions are simple reactions in which both the reactants and the products remain unchanged; under these conditions the degradation reactions should only be affected by the temperature while remaining completely

unaffected by the thermal history (i.e., from the momentary state). However, this scenario only occurs if the reactions have a fixed kinetic order at each temperature within the experimental range (Corradini et al., 2004; Peleg et al., 2004), which does not occur for the complex reactions involved in the degradation of quality indexes (i.e., enzymatic browning) of vegetable tissues. Under real conditions, the substrates for the degradative reactions are affected by the incidental conditions of the vegetable (i.e., pH, enzymatic activity, water activity, phenols content, etc.) in addition to the temperature. In particular, this scenario holds for fresh-cut products in which the degradation reactions are affected by the evolving gas composition inside the packages, which is also affected by the temperature. Moreover, the cold chain may be discontinued; Taoukis (2013) revealed that the temperature during distribution and storage may vary from 0 and 13°C, while in domestic refrigerators, the temperatures may vary from ~4 to ~11°C. Vergara et al. (2010) performed a study in Northern Italy, revealing that approximately 60% of home refrigerators ranged in temperature between 4.1 and 10.0 °C; only 30 % exhibited a temperature between 2-4°C.

To improve the estimations of shelf life, an alternative point of view involves treating the degradation reactions as cumulative forms of the temporal distribution of mortality events (van Boekel, 2002). In particular, van Boekel revealed that the microbial survival curve can be accurately described using the cumulative form of the Weibull distribution, while Corradini et al. (2004) proved that this alternative approach may describe the changes in several degradation reactions involving nutrients, pigments and enzymes. In addition, these authors proposed using a log-

logistic model rather than the conventional Arrhenius equation to describe the temperature dependence of the rate constant. This approach has been used to study the kinetic degradation of the vitamin C in green peas and spinach, the riboflavin in spinach, the thiamin in red gram split, the chlorophyll a in spinach puree, the polyphenol oxidase (PPO) in potato homogenate, and the vitamin C in strawberry juice (Corradini et al., 2006; Derossi et al., 2010). The Weibull model has also been used to describe the chemical and sensorial changes of fresh-cut produce (Iqbal et al., 2005; Oms-liu et al., 2009; Odriozola-Serrano et al., 2009); however, few studies are available regarding the estimated shelf life of fresh-cut rocket (Koukounaras et al., 2007), none of which utilize the Weibull-logistic model.

The aim of this work was to improve the management of the critical control points and quality maintenance while improving the estimation of the shelf life of fresh-cut rocket. This is important for planning produce logistics with fully automated distribution steps and better manage stocks, according to thermal history and, possibly, to priorities of potential customers.

More specific objectives are the use of the Weibullian-log logistic model to fit the changes of important sensorial, physical and chemical attributes of rocket leaves as affected by storage time and temperature, and the definition of the limiting factor of shelf life when several quality attributes are contemporaneously taken into account, and how this may be affected by changes in temperature.

5.1.3 Materials and Methods

5.1.3.1 Theoretical background

According to Corradini et al. (2004), the degradation reactions can be analyzed using the cumulative form of the Weibull distribution:

$$F(t) = \exp(-bt^n) \quad \text{Eq .1}$$

where *F(t)* is the fraction of the molecules that retain their activity after time t, and *b* and *n* are model constants. In the present study, several physical, chemical and sensorial quality attributes were evaluated based on the fraction molecules that retain their "intact" form, which indicates their initial quality. For example, according to Corradini et al. (2006) and Jiang et al. (2012), we can consider the ascorbic and dehydroascorbic acids the "intact" and "failure" molecules, respectively. Generally, this approach may be applied for all analyzed quality attributes. Consequently, because $F(t)$ may be expressed as $C(t)/C_0$, where $C(t)$ and C_0 are the amounts of "intact" molecules after times t and at $t = 0$, respectively, the degradation reactions may be reported as follow:

$$C(t)/C_0 = \exp[-b(T)t^{n(T)}] \quad \text{Eq. 2}$$

where *b(T)* and *n(T)* are temperature-dependent coefficients.

In Eq. 2, *n(T)* is the "shape factor", while the reciprocal of *b(T)* is the "location factor". Nevertheless, as reported by Corradini et al. (2004, 2005, and 2006), the *b* value has rate units (1/t), and therefore, Eq. 2 is considered a kinetic model. The temperature dependence of *b(T)* and *n(T)* was described by several authors through a log-logistic model (Peleg et al., 2002; Corradini et al. , 2004; Corradini et al., 2005; Corradini et al., 2006; Corradini et al., 2007) as:

$$b(T);\ n(T) = \log_e\{1+\exp[k(T-Tc)]\} \qquad \text{Eq. 3}$$

where Tc (°C) marks the critical temperature at which the degradation intensifies, and k ($°C^{-1}$) is the acceleration of the process beyond Tc. The b, n, k and Tc values were estimated via the least squares method using the Curve Fitting Toolbox of Matlab ver. 6.5 (MathWorks Inc, USA, 2002). However, as reported from Corradini et al. (2006), Eq. 3 is an empirical model, and other equations could be used. This was the case of texture score for which the temperature dependence of the b parameters of Weibull equation was modeled with the following *ad-hoc* empirical model:

$$b(T) = a*\exp(-kT^c) \quad \text{Eq. 7}$$

where a (1/t) is the b value when temperature is 0°C , k (1/T) is the constant which marks the increase of b values as a function of temperature, T (°C) is the storage temperature and c is a constant parameter (dimensionless). In this case, a different function describing the momentary degradation rate it is easily obtained by substituting Eqs. 3 and 7, respectively for n and b parameters, into equation 4.

5.1.3.2 Estimated shelf life under isothermal and non-isothermal conditions

When assuming that the storage occurs under non-isothermal conditions described by a general temperature profile $T(t)$, the momentary isothermal degradation rate may be obtained from the derivative of Eq.2 (Corradini et al., 2006):

$$d(C(t)/C0)/dt = -b(T)\exp[-b(T)t^{n(T)}]n(T)t^{n(T)-1} \quad \text{Eq. 4}$$

In eq. 4, $dC(t)/C_0dt$ is the momentary degradation rate at the momentary temperature $T(t)$ at time $t*$, which corresponds to the momentary concentration Ct:

$$t^* = \left\{ \frac{-Log_e \left[{}^{C_{(t)}}/_{C_0} \right]}{b(t)} \right\}^{1/n(t)} \quad \text{Eq. 5}$$

Because $b(t)=b[T(t)]$ and $n(t)=n[T(t)]$ under any temperature profile, Eqs. 3, 4 and 5 can be combined to create a very complicated degradation rate equation. However, when $b(T)$ is accurately described by Eq. 3 and n is not affected by the temperature (fixed n), the following can be obtained:

$d(C(t)/C0)/dt=\{-\log_e[1+\exp(k(T(t)-Tc)]\}\exp\{[-\log_e[1+\exp(k(T(t)-Tc)]t^{*n}]\}nt^{*n-1}$ Eq.6

Where t^* is as reported in Eq. 5.

Eq. 6 is an ordinary differential equation that may be solved using any software for any continuous or discontinuous temperature profile $T(t)$. However, according to Corradini et al. (2006), we approximated Eq. 6 through a difference equation that was solved using Microsoft Excel.

The goodness of fit was evaluated using the correlation coefficient, p-level and confidence intervals. In addition, the root mean square error (RMSE) value was calculated to evaluate the accuracy of the model (Togrul et al., 2002; Bas et al., 2007).

5.1.3.3 Sample preparation and experimental design

Fresh rocket leaves were purchased from a local grower (Foggia, Italy) and washed in a free chlorine solution (0.01% v/v) before being drained, portioned into 75 100-g samples that were packaged in PP bags (17.50 of size and OTR = 1800 $cm^3m^2d^{-1}$, WVTR = $6gm^2d^{-1}$). Twenty-five bags were stored at each temperature (0, 5 and 15°C); specifically, 3 bags for each of the 7 sampling times (0, 1, 2, 3, 6, 8, and 10

days) plus 4 additional bags for gas sampling. For the fresh samples, the sensory analysis and vitamin C (mg/100 g) data were assessed. The experimental data for each quality parameter were fitted using Eq. 2, and the estimated *b(T)* and *n(T)* for each storage temperature were fitted in Eq. 3 to estimate *k* and *Tc*. In a second experiment, non-isothermal conditions were used over 9 days of storage with a temperature profile [$T_1(t)$] in which 24 hours of temperature abuses was applied (13 °C) on days 2 and 6 of storage. Eighteen bags were stored under these conditions, assessing gas concentration and sensory analysis on 3 bags at 0, 1, 2, 5, 6, and 9 days of storage.

On each sampling day, the physical, sensorial and chemical quality attributes were measured. An analysis of variance (ANOVA) was performed to evaluate the significant differences ($p < 0.05$) in each quality attribute over time using the STATISTICA ver. 10 software (Statsoft, Tulsa, USA).

5.1.3.4 Physical and sensory attributes

The gas composition was tested before the bags were opened; additional bags equipped with rubber septa were assessed using a gas analyzer (Mapy 4.0, WITT, Germany).

A group of 4 laboratory-trained panelists subjectively assessed for appearance, green taste, pungency, color, texture and overall quality on a scale ranging from 5 to 1, where 5 denoted a typical attribute that is very intense and without defects, 4 denoted intense with slight defects, 3 denoted fair with accepTable defects, 2 denoted poor with major defects and 1 denoted inedible very poor with evident

defects. The off-odors were evaluated using a scale from 1 to 5, where 1 denoted no off odors and 5 denoted very intense off-odors. The overall quality was described to panelists as a global acceptance of the product that incorporated the visual, textural, and taste attributes. In general, a score of 3 was the limit of marketability (with the exception of firmness, where samples that were too firm may not be accepted by the consumers) and 2 as the limit for edibility.

5.1.3.5 Vitamin C determination

Ten grams of fresh tissue were homogenized using 10 mL of MeOH/H_2O (5:95) plus citric acid (21 g L−1) with EDTA (0.5 g L^{-1}). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). The ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata et al. (1992) with some modifications. The HPLC analysis was undertaken after derivatizing DHAA with 1,2-phenylenediamine dihydrochloride (OPDA) to form 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ). The samples (20 µl) were analyzed using an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, and a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). The DFQ and AA were separated on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH

4.5. The flow rate was 1 ml min−1. The AA and DHA contents were expressed as mg of ascorbic or dehydroascorbic acid per kg of fresh weight (mg kg^{-1}).

5.1.4 Results and Discussion

5.1.4.1 Characterization of the degradation kinetics of the rocket leaves

The results of the analysis of variance for the appearance, overall quality, color, off-odors, and texture scores (among sensorial parameters) vitamin C content, oxygen and carbon dioxide concentration ($p<0.05$) showed significant variations over the 10 days of storage at each temperature (data not shown).

To indicate whether a fixed or variable *n*-parameter may affect the goodness of the fit, Table 1 shows the estimated appearance score for the fresh rocket leaves stored at 0, 5 and 15°C. When the value of *n* was free to vary, the experimental data were fit well with correlation coefficients above 0.950 and RMSE lower than 0.0575. However, only slight differences were observed for the samples stored at 0, 5 and 15 °C with values of 1.25, 1.34 and 1.37, respectively. Therefore, a new fit was performed where $n = 1.32$; this value is the average of the previously observed values. The results revealed a comparable ability to fit the experimental data, generating correlation coefficients between 0.950 and 0.995 and RMSE below 0.0526. We used this second form of the Weibull model (with a fixed power *n*) because it simplified the data processing and shelf life predictions. Moreover, this value revealed that the appearance score for the fresh rockets leaves did not follow traditional first-order kinetics which generated lower correlation coefficients that ranged between 0.932 and 0.978 (data not shown). These results agree with those of

Corradini et al. (2004; 2006) and Amodio et al. (2013): the Weibull model can describe degradation reactions better than the conventional kinetic models due to its flexibility.

As known, when *n* equals 1 the Weibull model corresponds to a first-order kinetic, a degradation of a single chemical species may be assumed on the basis of the rules of chemical kinetics (Corradini et al., 2006). Our results showing a fixed *n* of 1.32 allowed hypothesizing the existence of a more complex mechanism with the involving of several chemical species in the degradation of visual aspect of rocket leaves. Furthermore, because a fixed *n* was useful when describing the decay in the appearance at all of the studied temperatures, the mechanism of degradation would seem to be not dependent from the temperature in the range of 0 and 15°C. When accounting for the estimated *b* values, the degradation rate increased with the temperature; however, considering the confidence intervals, significant differences were only observed between 5 and 15 °C (Table 1). In particular, at these temperatures, values of 0.0439 and 0.0849 d^{-1} were estimated, indicating a ~1.9-fold increase in the degradation rate of the appearance score.

Figure 1 shows the changes in the appearance score of fresh rockets stored at 0, 5 and 15°C over time. According to Table 1, the experimental data and the fits obtained when *n* was 1.32 were practically overlapping, indicating that the model precisely accounted for changes in appearance over time.

Table 2 shows the results of the fit performed with variable or fixed *n* values for the quality attributes and the gas concentrations; in addition, the goodness of fit measures obtained with the traditional first-order kinetics and the Weibull model is

shown. In all cases, the Weibull model improved the fit of the experimental data with correlation coefficients ranging between 0.964 and 0.993; when using the traditional first-order kinetic, values between 0.899 and 0.986 were obtained.

Therefore, the Weibullian-log logistic model may be extended to all of the quality attributes studied in addition to the changes in the gas composition over time, which strongly depended on the temperature (Jacxsens et al., 2000; Waghmare et al., 2013). Nevertheless, for CO_2 concentration, according to Eq. 2, *C(t)* is higher than *C0* since CO_2 is developed over time. The same consideration can be done for the off-odor score (1=no and 5=very intense off-odors).

For the overall quality, using a variable *n* enabled the best fit with the experimental data. In particular, *n* values of 0.654, 0.885 and 1.539 were estimated for samples stored at 0, 5 and 15 °C, suggesting a complex reaction mechanism for the degradation of this attribute. Moreover, the significant change in the *n* values with the temperature allows for speculation regarding a change in the type and/or the number of reactions involved when the temperature increases from 0 to 15 °C. Similar results were obtained for the off-odors score, the texture score, the vitamin C content and the concentrations of both oxygen and carbon dioxide, for which an increase in the *n*-parameter was observed with storage temperature. For the color score, a fixed *n* of 1.348 fit experimental data better at all studied temperatures.

When accounting for the estimated degradation rates (*b*) in Table 2, an expected increase with the temperature was observed for color scores of 0.015, 0.019 and 0.047 d^{-1}, respectively at 0, 5 and 15°C; unclear temperature dependence was observed for the other quality attributes.

However, when the values of the *n*-parameter change with the temperature, the degradation rates cannot be compare directly because the Weibull model may assume very different shapes for different *n* values. As reported by several authors, the degradation rate is a decreasing function when $n < 1$ and an increasing function when $n > 1$ for the Weibull model (Cunha et al., 1998). Obviously, under these conditions, a common increase in the rate constant versus the temperature cannot be expected.

To explain the changes in the quality attributes over time, the changes in the overall quality score of rocket leaves stored at 0, 5 and 15°C and the fits obtained using Weibull model are shown in figure 2. According to the results of Table 2, a good agreement between the experimental data and the fits was observed. Samples stored at 0 and 5°C, exhibited only slight differences during storage, generating, respectively, values of 0.786 and 0.795 after 3 days. This is because the estimated n values were not statistical different leading to a two degradation curve practically overlapped. For the samples stored at 15°C, after a slight variation during the first two days a sudden drop was observed, reaching values of 0.702 after 3 days and 0.272 after 7 days.

Figure 3 shows the changes in the vitamin C content and the fits for the rocket leaves at 0, 5 and 15°C during storage. In this case, the Weibull model exhibited a correlation coefficient above 0.970 and a RMSE below 0.03891 (Table 2) while by using the common first-order kinetic correlation coefficient in the range of 0.92 and 0.95 were observed proving again as the Weibull model allowed to better fit the experimental data.

Moreover, in this case a significant difference in vitamin C concentration was observed between 0 and 5°C with values of 0.954 and 0.864, respectively, after 3 days; a value of 0.483 was obtained for the samples stored at 15°C.

The effect of the temperature on parameters *b* and *n* of the Weibull model and the results of the fitting procedures performed using the log-logistic model, are shown in Table 3. In almost every case, the equation described the temperature dependence of both *b* and *n* well, obtaining correlation coefficients ranging between 0.955 and 0.999 and RMSE values below 0.12. Similar results were obtained for the oxygen and carbon dioxide; for these parameters, the log-logistic model described the variations in *b* and *n* with sufficient precision, generating correlation coefficients between 0.910 and 0.999.

The dependence of the Weibull parameters on temperature was fitted using a log logistic model, which allows obtaining the corresponding *k* and *Tc* values for each quality attributes. The obtained values were subsequently incorporated into the degradation rate model (Eq. 6) and solved to obtain estimation under non isothermal conditions for each quality index. Based on these results, the assumption of a unique activation energy, which is essential when using the Arrhenius model, is not required.

However, as reported by Corradini et al. (2006), the log-logistic model is only one of the possible models that can be used to describe the temperature dependence of the Weibull parameters. Figure 4 shows, the temperature dependence of the *b* parameter for the appearance score obtained after fitting the b values with Eq. 3; according to Table 3, the model adequately fit the *b* values, showing a correlation

coefficient of 0.985 and a RMSE of 0.057; in addition, the estimated *k* and *Tc* parameters of 0.0059 d^{-1} and 55.64 °C, respectively, may be used to calculate the degradation rates at any temperature inside the examined range.

5.1.4.2 Shelf-life estimation and definition of the limiting factors

Once the abilities of the Weibull and log-logistic models were proven to fit the experimental data, these models may be used to estimate the shelf life of fresh rocket.

Because the limit of marketability for the appearance score (score 3), which corresponds to a $A_t/A_0 = 0.6$, a end of shelf life of 7.3, 5.8 and 3.7 days can be estimated for samples stored at 0, 5 and 15 °C, respectively (from the fit in Figure 1).

When the texture score is used as the attribute that defines the shelf life limit (where a score of 3 is the limit of marketability), values of 12.6, 10.4 and 3.1 days can be estimated at 0, 5 and 15 °C, respectively, showing that appearance reached an unaccepTable value faster than the texture at 0 and 5 °C (data not shown). Piagentini et al. (1995) also monitored the major sensory changes of three freshly cut leafy vegetables (Iceberg and Romaine lettuce and chicory) between 2 to 20 °C, finding that the limiting quality factor at any temperature was the general appearance. The same authors reported that for chicory, any of the attributes could be considered the shelf-life limiting quality attribute between 15 and 20° C, indicating a general acceleration in the degradation at higher temperatures, although they did not monitor any chemical attributes.

For the ascorbic acid loss, the number of days needed to reach a content of 20 mg $100g^{-1}$, (which is the limit for shelf-life because this value corresponds to 50% of the recommended daily intake according to the Australia and New Zealand Food Authority, (2001), are estimated to be 13.5, 7.2 and 2.4 d, respectively, at 0, 5 and 15°C. While at 0 and at 5 °C the visual aspect degraded faster than vitamin C, vitamin C at 15°C reached the marketability limit when appearance score was still accepTable (3.8) after comparing these values with the shelf-life based on appearance.

For the off-odor score, a value of 3, which is also the reference limit for marketability, was reached 12.2, 9.5 and 4.5 days r at 0, 5 and 15°C, respectively. Therefore, the appearance score was the limiting factor for shelf life from 0-5°C, but at 15°C, shelf life would be limited by the ascorbic acid content, followed by the texture because the degradation rates of these attributes increase with temperature more than the appearance and off-odor.

These differences are shown in Figure 5, in which the shelf life estimated by the Weibull log-logistic model, was fitted as a function of storage temperature. In particular, a linear model well described the changes of shelf life in terms of appearance, texture and off-odor scores obtaining a correlation coefficient greater than 0.99 while for ascorbic acid an exponential decay explained the changes in the shelf life attributed to the temperature changes ($r = 0.97$). Then, to define the effect of the storage temperature on the shelf life of fresh rocket leaves, the Temperature Unit (TU) parameter, which is the increase in temperature that reduces the shelf life by 1 day, was introduced. When the shelf life is calculated based on the appearance,

texture and off-odors scores, the TU values are 4.4, 1.6, 2.0, respectively; when the shelf life is considered relative to the ascorbic acid loss, the TU is approximately 1.5, meaning that a slight increase in the temperature (1.5°C) can induce the loss of 1 day of shelf life. The thermal sensitivity of vitamin C is well known; the temperature is one of the most important postharvest factors affecting the final vitamin C content in fresh commodities (Lee et al., 2000), such as berry fruits (Agar et al., 1997), strawberries, persimmons (Palmer Wright et al., 1997), spinach (Gil et al., 1999), and purslane (Rinaldi et al., 2010). Figure 5 shows that the appearance score limits the shelf life of fresh rocket leaves stored below 7 °C, while at higher temperatures, vitamin C is the limiting factor.

The changes in the shelf life as a function of storage temperature cannot be based only on a temperature effect. In particular, for the degradation reactions in living tissue, such as fresh rocket leaves, several variables in the vegetables (i.e., pH, enzymatic activity, water activity, phenol content, etc.) and the gas composition inside the packages can significantly affect the degradation rates of each quality attributes. Therefore, the changes in the oxygen content inside the packages over time and the fit obtained using Weibull model are shown in Figure 6. This figure shows that the oxygen fractions of 11.3, 8.6 and 0.7% at 0, 5 and 15°C were estimated when the limit for marketability based on the appearance score was reached. Under the same conditions and while using the parameters of the Weibull model listed in Table 2, carbon dioxide fractions of 7.9, 8.5 and 10.9% at 0, 5 and 15 °C were estimated. The large differences observed between the temperatures for oxygen depletion and CO_2 accumulation can be attributed to the dependence of the

respiration rate and gas permeability of the film on the temperature (Exama et al; 1993; Jacxsens et al., 2000; Waghmare et al. 2013)

5.1.4.3 Predicted shelf life under non-isothermal conditions

When a constant storage temperature during a cold chain is not guaranteed, the degradation reactions do not follow the behavior shown in figures 1 and 2, and the factors limiting the shelf life can vary significantly relative to the temperature profile imposed upon the leaves.

In particular, for the quality attributes, which showed that the *n*-parameter is temperature-dependent using the Arrhenius equation to estimate the shelf life under non-isothermal conditions can produce significant discrepancies between the estimated and experimental results.

By inserting temperature variations during storage as a mathematical expression (*T(t)*) in the Eq. 6 the momentary degradation rate of any quality attribute and/or gas concentration can be obtained, enabling estimations of the shelf life under non-isothermal conditions.

For example, Figures 7a and 7b show the estimated values and the experimental data for the changes in the oxygen and carbon dioxide concentrations inside the packages of fresh rocket leaves stored under isothermal (5°C) and non-isothermal conditions. As expected, the oxygen concentration suddenly decreased when the temperature increased to 13°C. After 2 days, the estimated "intact" oxygen molecules accounted for 10.8 and 15.3 % of the total, respectively, under non-isothermal and isothermal conditions; after 6 days of storage, values of 2.2 and

7.93% were estimated. Similarly, an increase in the carbon dioxide concentration was observed under non-isothermal conditions, reaching values of 4.7, 6.28 and 9.43, respectively, after 2, 3 and 6 days. After comparing the estimated and measured values, the Weibullian-logistic model could predict the changes in the O_2 and CO_2 concentrations under non-isothermal conditions with sufficient precision, obtaining a maximum error of 2.2 % and 0.52%, respectively, for oxygen and carbon dioxide.

Figure 8 shows the changes of appearance and color scores when the rocket leaves were stored under non-isothermal conditions as a function of storage time. In both cases the experimental data generally follow the estimated curves in non-isothermal conditions although discrepancies appeared after the second period at 13°C, particularly for score appearance. These differences could be the results of a change of degradation patter due to fluctuation temperature. Furhtermore, the biological variation, as extensively proved by Hertog et al. (2007) could have increased the difference between experimental and estimated behavior. However, taking into account the appearance score, the limit of marketability was reached in 5.25 days whereas 5.8 days were necessary when the rockets were stored in isothermal condition at 5°C.

By simulating the shelf-life as changes in vitamin C occurring with this temperature profile, the limit for ascorbic acid (50% of the RDI) would be reached only after 4.2 days under non-isothermal conditions (data not shown), compared to 7.2 at 5 °C. At end of the shelf life defined by the ascorbic acid degradation (4.2 days) at 5°C, an appearance score of 3.4 was estimated, showing that the rocket leaves were

still above the limit of marketability for appearance. This scenario occurred because, according to the previously reported TU values, the temperature dependence of the degradation rate was higher for ascorbic acid than for the appearance. Therefore, when the shelf-life is estimated under isothermal conditions and a temperature abuse occurs during distribution, the potential error is attributed to the increased degradation rate for the limiting quality factor during the temperature abuse to the fact that a different quality attribute could limit shelf life. Based on these consideration, the effect of a situation in which rockets leaves are exposed to temperature continuously fluctuating between 5 and 10°C was estimated for the visual appearance score (figure 9). This parameter was considered based on the work of Tauokis (2013), who showed that the temperature inside a domestic refrigerator varies continuously.

A temperature oscillation of 5°C greatly affects the changes in the appearance score; after 1 day, this parameter fell below the estimated value for the isothermal conditions. In particular, the limit of marketability was reached after 5.0 days, exhibiting an unexpected reduction of almost 1 day relative to the samples stored at 5°C.

5.1.5 Conclusion

Knowing the fate of the external and internal quality attributes of ready-to-eat produce is critically important when considering the logistic aspects of distribution. Estimating the shelf life and defining the most limiting attributes enable better stock management and satisfaction for potential customers. Toward these objectives, the

degradation kinetics of the most important quality attributes for fresh-cut rocket leaves over time were studied based on the cumulative forms of the survival curves for the molecules involved in the degradation reactions. In particular, the Weibull model fit the experimental data, demonstrating that the changes in the quality of freshly rocket are rarely described adequately by conventional first-order kinetics. Additionally, the temperature dependence of the Weibull parameters *b(T)* and *n(T)* could be fitted with a log-logistic model that accounts for the thermal history of the products. Therefore, the traditional Arrhenius model, which assumes that simple chemical reactions may be characterized from a single activation energy, may be unnecessary for freshly rocket.

In general, when fresh-cut rocket leaves were stored at constant temperatures, appearance score limited the shelf life, reaching the limit of marketability after 7.3, 5.8 and 3.7 days, respectively, for samples stored at 0, 5 and 15°C; nevertheless, an increase in the temperature affected the loss of ascorbic acid loss more than the appearance and off-odor scores, allowing this nutritional attribute to limit the shelf life. During non-isothermal storage, the degradation of each quality index may show different behaviors over time, allowing the limiting factor of the shelf life definition to vary. Finally, a Weibullian-logistic model was useful when predicting the shelf life of freshly cut rocket leaves under isothermal and non-isothermal conditions; in particular, any temperature profile $T(t)$ detected along the entire cold chain could be used to define the “best before” date reported on the packages better in the second case.

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5.1.6 References

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Table 1. Weibull parameters for the appearance scores of fresh rocket leaves stored under a passive MAP at different temperatures.

Temperature (°C)	$b(d^{-1})$	Confidence interval	n	Confidence interval	r	RMSE
Variable n(T)						
0	0.043	0.007 – 0.078	1.25	0.86 – 1.60	0.976	0.0357
5	0.041	0.009 – 0.094	1.34	0.73 – 1.96	0.950	0.0575
15	0.079	0.040 – 0.100	1.37	1.16 – 1.57	0.996	0.0211
Fixed n = 1.32						
0	0.0381	0.032 – 0.0434	1.32	-	0.975	0.0331
5	0.0439	0.034 – 0.0531	1.32	-	0.950	0.0526
15	0.0849	0.077 – 0.0921	1.32	-	0.995	0.0198

Table 2. Weibull parameters for the quality indexes of the fresh rocket leaves stored at 0, 5 and 15°C. r* is the correlation coefficient obtained by fitting experimental data with a conventional first-order kinetics equation.

Temperature (°C)	b (d^{-1})	Confidence interval	n	Confidence interval	r	RMSE	r*
Quality attributes							
Overall quality score							
0	0.117	0.075 – 0.159	0.654	0.465 – 0.843	0.988	0.0452	0.948
5	0.086	0.045 – 0.127	0.885	0.643 – 1.126	0.989	0.0645	0.986
15	0.065	0.012 – 0.118	1.539	1.002 – 2.07	0.992	0.0421	0.960
Color score							
0	0.015	0.012 – 0.017	1.348	-	0.980	0.0170	0.980
5	0.019	0.016 – 0.022	1.348	-	0.964	0.0244	0.902
15	0.047	0.035 – 0.076	1.348	-	0.982	0.0762	0.899
Off-odors score							
0	$3.9*10^{-4}$	$2.8*10^{-4} – 4.7*10^{-4}$	2.84	1.83 – 4.51	0.975	0.089	0.956
5	0.0018	0.0010-0.0025	2.62	1.83 - 3.4	0.990	0.024	0.978
15	$2.4*10^{-5}$	$1.2*10^{-5} – 7.8*10^{-5}$	6.39	3.54 - 9.21	0.990	0.012	0.956
Texture score							
0	0.082	0.080 – 0.089	0.719	0.650 – 0.753	0.980	0.0170	0.980
5	0.074	0.070 – 0.081	0.819	0.789 – 0.923	0.964	0.0244	0.902
15	0.069	0.060 – 0.075	1.436	1.230 – 1.654	0.982	0.0762	0.899

Vitamin C							
0	0.0087	0.043 – 0.012	1.530	1.230 – 1.752	0.970	0.01778	0.965
5	0.0342	0.023 – 0.042	1.317	1.023 – 1.534	0.985	0.02429	0.970
15	0.0702	0.065 – 0.082	2.129	1.893 – 2.204	0.985	0.03891	0.942
			Gas composition				
Oxygen concentration							
0	0.095	0.08 – 0.142	0.844	0.86 – 1.60	0.972	0.0265	0.970
5	0.153	0.121 – 0.201	1.084	0.73 – 1.57	0.976	0.0299	0.964
15	0.373	0.302 – 0.401	1.661	1.16 – 1.96	0.993	0.0268	0.983
Carbon dioxide concentration							
0	0.199	0.079 – 0.219	1.169	0.756 – 1.581	0.973	0.06468	0.965
5	0.195	0.118 – 0.232	1.166	0.90 – 1.432	0.989	0.04211	0.976
15	0.562	0.439 – 0.931	1.923	0.436 – 2.52	0.984	0.06597	0.945

Table 3. Log-logistic parameters estimated for the different sensorial, physical and chemical attributes of freshly cut rocket leaves stored under a MAP at different temperatures

logistic parameters	*K*	Confidence interval	*Tc*	Confidence interval	r	RMSE
Quality attributes						
			Appearance score			
b(T)	0.059	0.005 – 0.015	55.64	44.8 – 60.5	0.985	0.057
			Overall quality score			
n(T)	0.093	0.071 – 0.115	1.029	-1.423 – 3.48	0.999	<0.001
			Color score			
b(T)	0.083	0.003 – 0.178	51.64	23.4 – 56.7	0.993	0.0019
			Off-odors score			
n(T)	0.267	0.130-0.432	6.23	4.52 – 8.34	0.955	0.0023
			Texture score			
b(t)	a=0.082 b=0.043 n=0.522	0.080 – 0.083 0.042 – 0.047 0.048 – 0.054	-	-	0.999	<0.001
n(T)	0.079	0.097 – 0.156	0.623	-9.51 – 10.75	0.988	0.023
			Vitamin C			
b(t)	0.099	-0.310 – 0.510	41.25	30.04 – 55.22	0.969	0.09
n(t)	0.057	-0.404 – 0.519	-18.19		0.843	0.12
Gas composition						
			Oxygen Concentration			
b(T)	0.072	0.025 – 0.118	2.34	1.85 – 8.31	0.996	0.0232
n(T)	0.078	0.077 – 0.078	-3.61	-3.653 – -3.581	0.999	<0.001
			Carbon dioxide concentration			
b(T)	0.199	0.079 – 0.2192	1.169	0.756 – 1.581	0.973	0.0647
n(T)	0.072	-0.214 – 0.358	-3.85	-80.51 – 62.82	0.910	0.1851

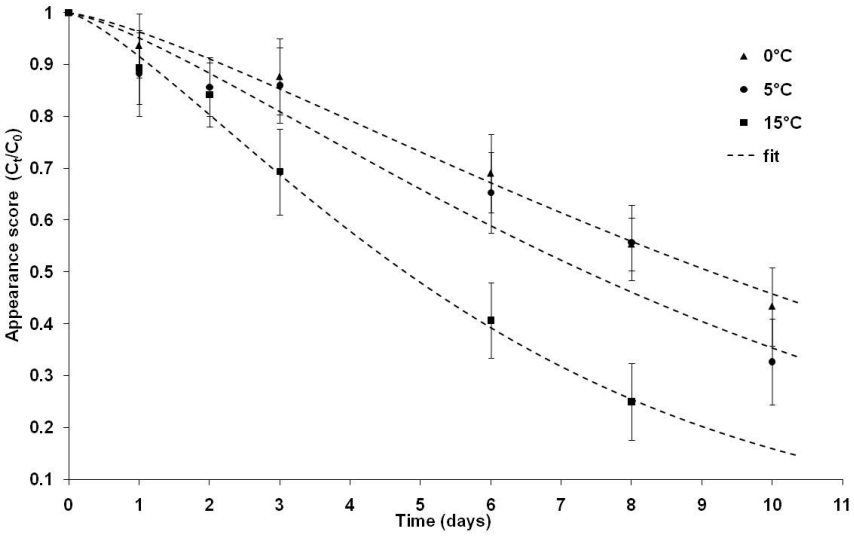


Figure 1. Changes in the appearance score of the freshly cut rocket leaves stored under a passive MAP at 0, 5 and 15°C over time. Error bars indicate the standard deviations of the mean values.

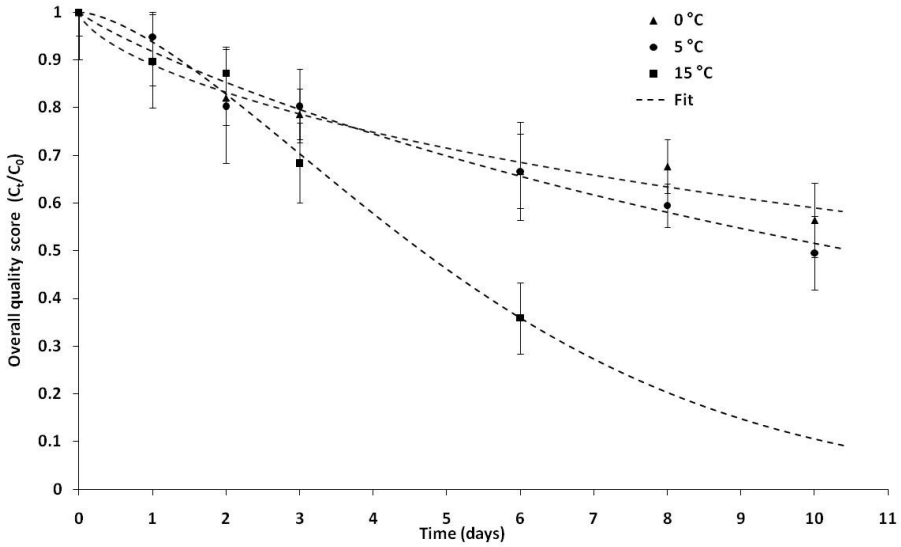


Figure 2. Change in the overall quality score of fresh rocket leaves stored under a passive MAP at 0, 5 and 15 °C over time. Error bars indicate the standard deviations of the mean values.

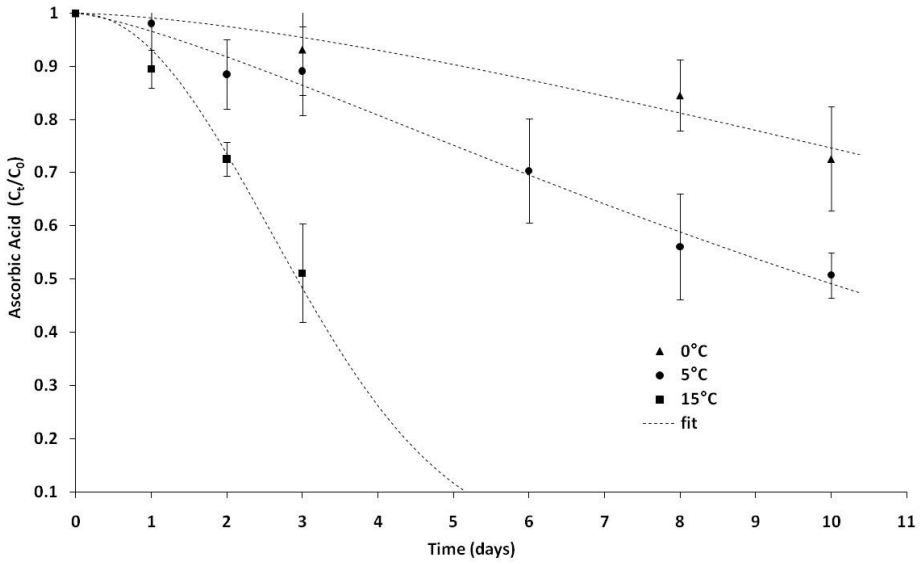


Figure 3. Changes in the ascorbic acid content of fresh rocket leaves stored at 0, 5 and 15°C under a MAP over time. Error bars indicate the standard deviations of the mean values.

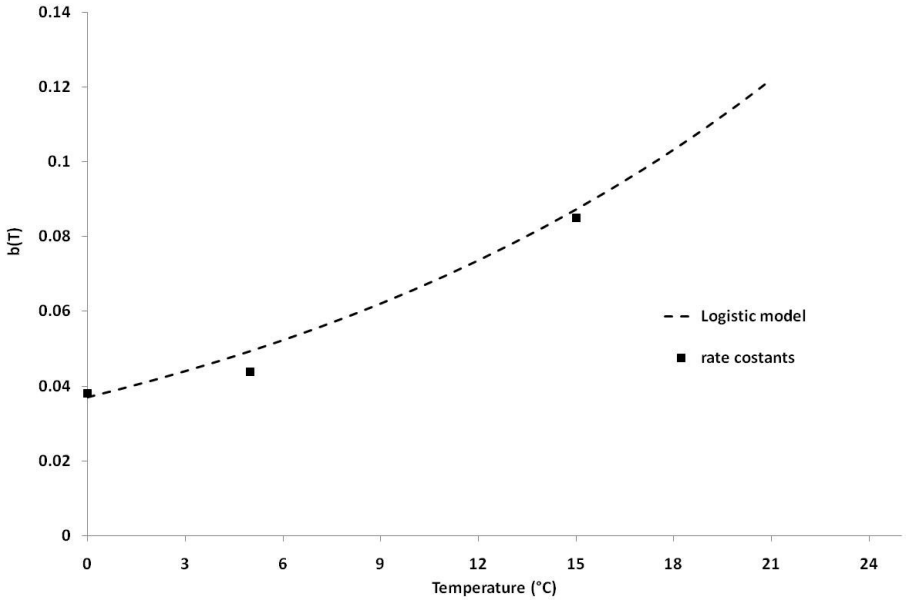


Figure 4. Temperature dependence of the Weibull parameter (*b*) for the appearance score of fresh rocket leaves stored at 0, 5 and 15°C.

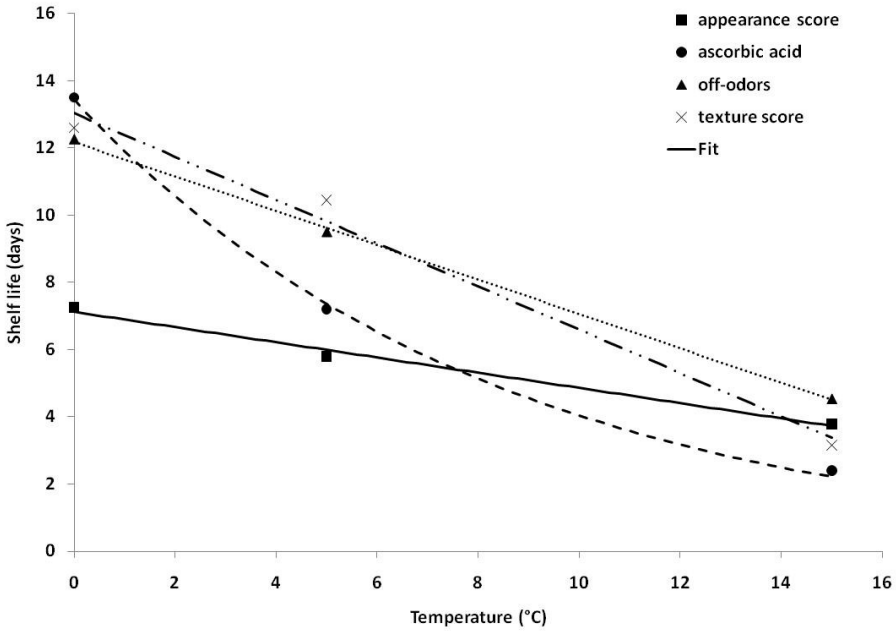


Figure 5. Estimated shelf life for different quality indexes of fresh rocket as a function of the storage temperature.

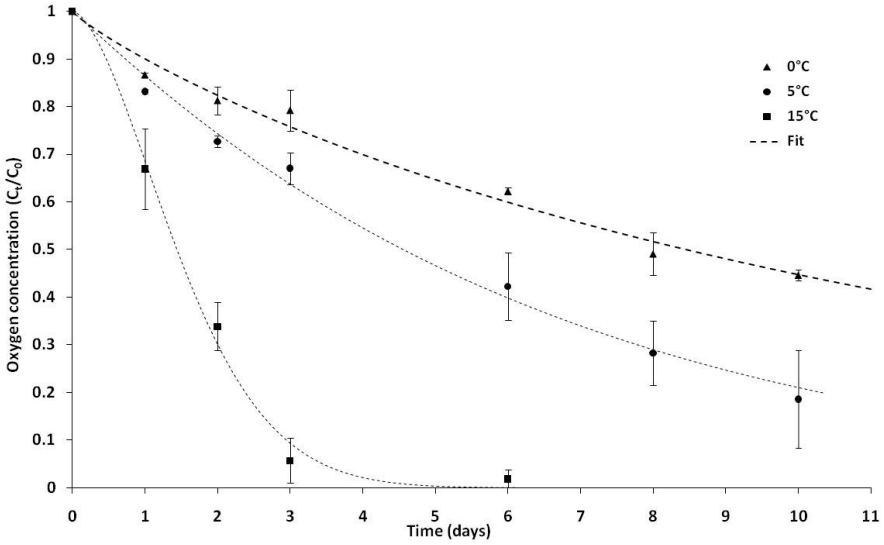
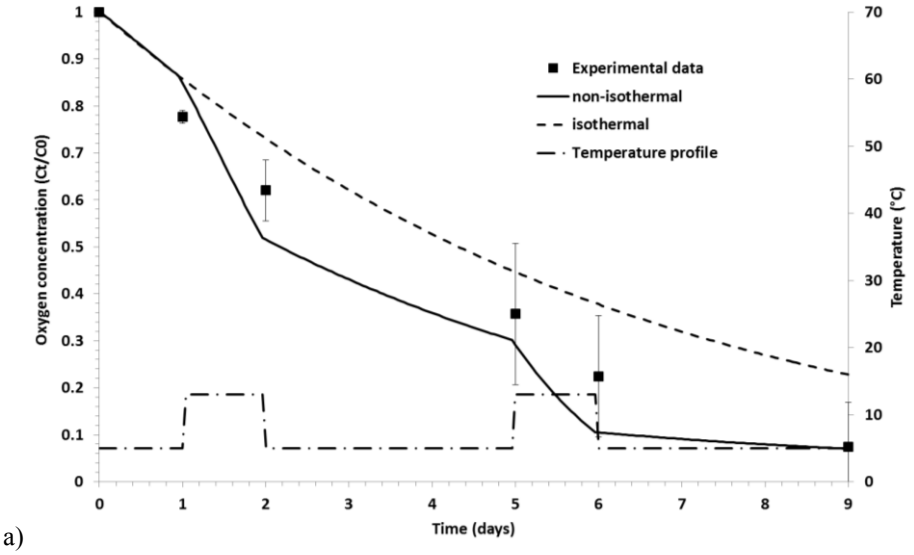
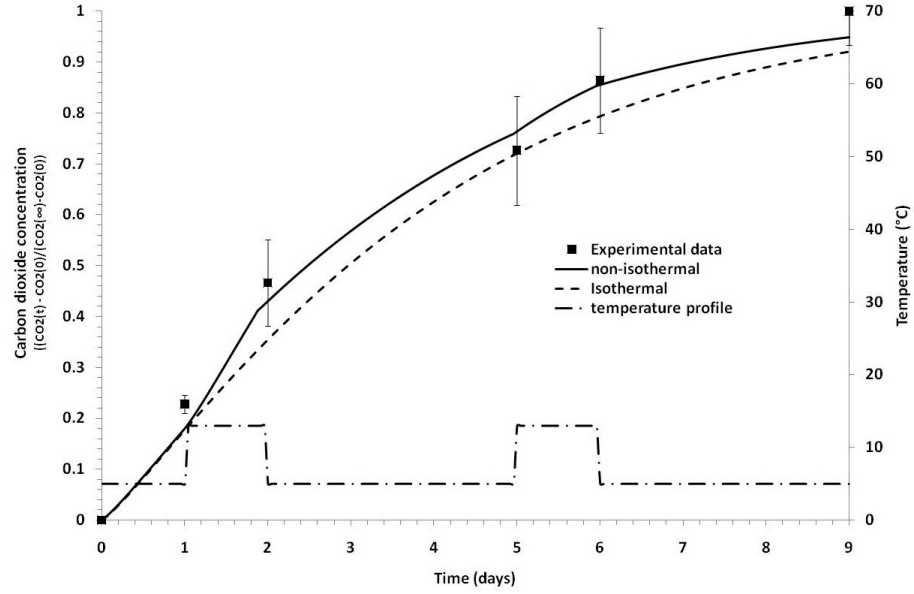


Figure 6. Evolution of the oxygen concentration inside packages of fresh rocket leaves stored at 0, 5 and 15°C. Error bars indicate the standard deviations of the mean values.



a)



b)

Figure 7. Changes in the gas composition of packages containing fresh rocket leaves stored at 5°C under non-isothermal condition. a) Variation in the oxygen concentration; b) variation in the carbon dioxide concentration. Error bars indicate the standard deviations of the mean values.

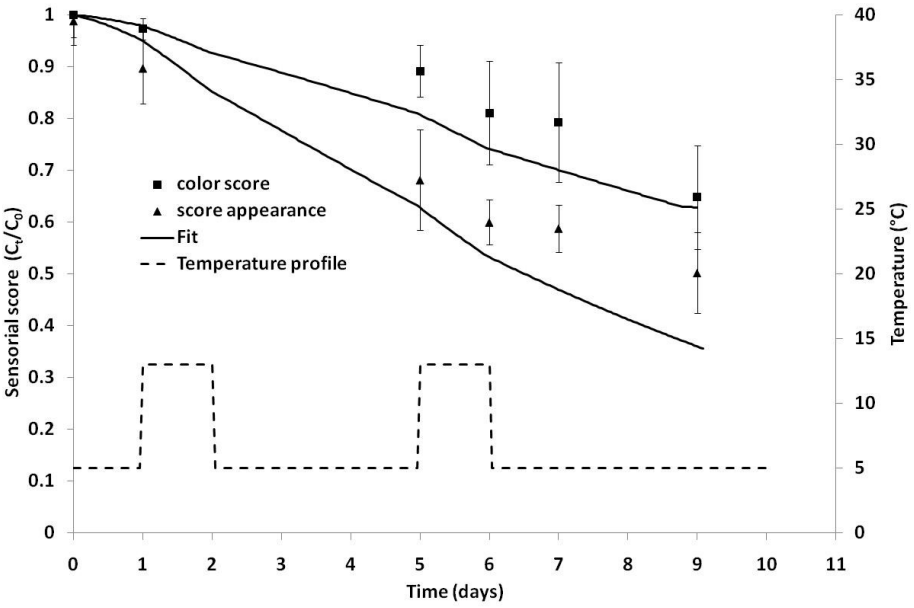


Figure 8. Changes in the appearance and color scores of fresh rocket leaves stored under non-isothermal condition over time. Error bars indicate the standard deviations of the mean values.

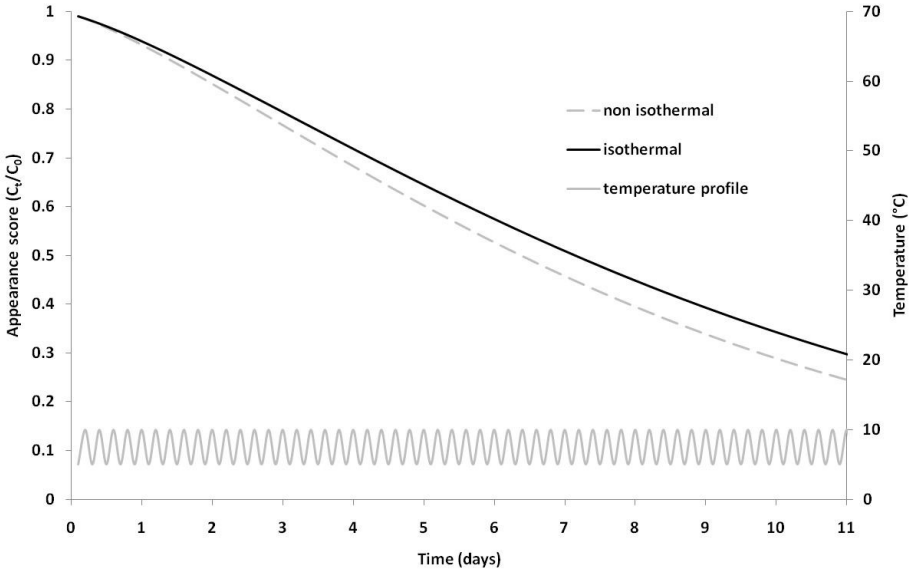


Figure 9. Predicted appearance score changes for the fresh rocket leaves stored at 5°C under non-isothermal conditions over time.

6.1 EVOLUTION OF VOLATILE PROFILE OF FRESH ROCKET LEAVES AS AFFECTED BY MODIFIED ATMOSPHERE PACKAGING (MAP) IN ISOTHERMAL AND NON ISOTHERMAL CONDITIONS

6.1.1 Abstract

The aim of the study was to investigate the effect of MAP storage on sensory attributes and volatile composition of wild rockets (*Diplotaxis tenuifolia*). Fresh rocket leaves were packed in polypropylene (PP) bags and stored in isothermal (5°C) and non isothermal conditions in two different experiment. The first aimed to study the effect of MAP on sensory attributes and volatiles over the storage in air, whereas in the second one the effect of two temperature abuses (of 24hours) at 13°C, was investigated. Sensory attributes and volatiles were monitored at day 0 and during storage (8 and 9 days in isothermal and non isothermal conditions, respectively). Data were analyzed using Principal Components Analysis. Twenty-five volatiles were determined, including C6, C5, isothiocyanate, sulfur compounds and lipid derivative compounds through the headspace SPME GC-MS analysis. Loss of the main odor active volatile compounds and formation of off-flavors were detected and correlated with the storage time. Rocket stored in MAP showed a lower loss of volatile compounds responsible for the typical odor (thiocyanates, isothiocyanates) and production of off-odors (dimethyl sulfide, acetaldehyde) than the control in air. Visual quality was better preserved in rocket stored in MAP than in air. The rocket stored in non isothermal conditions showed an increased production of *off-odors* (acetaldehyde, dimethyl sulfide) soon after the storage at

high temperature (13°C,t2) and for all the experiment duration. Dimethyl sulfide and acetaldehyde could be an effective tool to track temperature fluctuation in the product history.

6.1.2 Introduction

Wild rocket (*Diplotaxis tenuifolia*) is a popular leafy vegetable in Europe, often used raw in salads, and therefore one of the most represented in the fresh-cut market. Rocket is generally stored refrigerated, as low temperature has been shown to reduce respiration rates and browning of fresh-cut vegetables (Koukounaras et al., 2007; Lokke et al., 2012); its typical shelf life is of 1-2 weeks.

Packaging of leafy vegetables in trays wrapped in polypropylene film or pouches, besides to facilitate produce handling, creates a modified atmosphere (MAP) in the package, which reduces loss of humidity, prevents spoilage, and prolongs shelf-life (Kalio, 2008, Kim et al., 2004; Sandhya, 2010; Smyth et al., 1998). Controlled atmosphere has been shown to preserve quality and phytonutrients of *Diplotaxis tenuifolia* (Martinez-Sanchez et al., 2006); however, MAP beneficial effects can be lost if the packaged product suffers the development of a too low O_2 and too high CO_2 concentrations eventually inducing the development of off-odors (Nielsen et al., 2008). Visual attributes (color, the absence of defected leaves and the swelling of packaging) and off-odors perceived soon after the bag opening are undoubtedly the most important sensory quality parameters which affect the consumer choice on the market. Therefore there is a needing of prevent undesiderable flavor of

packaged products, and to this aim understand how volatile profile may change in MAP packaging along the cold chain and in case of temperature abuse.

The characteristic pungent flavour of fresh rocket has been described to be related to glucosinolates and their breakdown products isothiocyanates (Bennett et al., 2006; Bennett et al., 2002; D'Antuono et al., 2009; Pasini et al., 2011). It is formed immediately after tissue disrupting when enzymatic reactions occur giving rise to the formation of a lot number of volatiles (Bones et al., 2006). The main constituent has been identified to be 4-methylthiobutyl isothiocyanate (erucin) (Miyazawa et al., 2002), reported to have antioxidant (Barillari et al., 2005) and inflammation-preventive activities (Yehuda et al., 2009). The hydrolytic products have been described as pharmaceutically more active than their parent glucosinolate molecule (Faulkner et al., 1998). However, the characteristic aroma is not the result of one single odor impression but of an array of compounds; indeed, several compounds were found to be of essential importance (Jirovetz et al., 2002). Hexanal, *trans*-2-hexenal, cis-3-hexen-1-ol, *trans*-2-hexen-1-ol were reported to be responsible for green aroma in Austrian *Eruca sativa* salad; benzaldehyde and *trans,trans*-2,4-heptadienal were responsible for nutty and almond-like odor notes. Some volatile compounds are more specific to mechanical injury, such as the C6 aldehydes and alcohols from lipoxygenase (LOX) action, which originate from membranes lipids, linoleic and linolenic acids (Arey et al., 1991); moreover, volatiles contribute to the formation of off-odours depending on their concentration (Poll et al., 2006). Nielsen et al. (2008) studied the effect of the packaging atmosphere on the development of off-odors focusing the investigation on DMS and DMDS compounds. Number and

proportions of *Eruca sativa* volatiles vary mainly in accordance with isolation methods including hydrodistillation-extraction by diethyl ether (Miyazawa et al., 2002), hydrodistillation in a Clevenger-type apparatus (Blažević et al., 2008), solid-phase microextraction (SPME) (Jirovetz et al., 2002). In particular, SPME has been demonstrated to be simple, rapid, solvent-free and effective for characterization purposes.

To the best of our knowledge, at present, no information is available on the evolution of wild rocket volatile profile, including both compounds responsible for typical scents and off-flavors. Therefore, this work aimed to track volatile changes in MAP stored wild rocket under isothermal (5°C) and non isothermal conditions, with the final objective of understanding the impact of temporary temperature abuses and if any volatile can be individuated as marker of improper storage conditions. Starting for these information, volatile sensors can be in fact further developed to help processor, distributors and final consumers to better track the quality of the products.

6.1.3 Materials and methods

6.1.3.1 Plant material and minimal processing

Fresh rocket leaves (*Diplotaxis tenuifolia*) were harvested in Salento (Apulia, Italy), washed in a free chlorine solution (0.01% v/v) before being drained, portioned into 50 g samples and packaged in PP bags (17.5x17.5 cm^2, OTR = 1800 $cm^3m^2d^{-1}$, WVTR = 6gm^2d^{-1}). In the first experiment 15 bags (3 replicates x 5 sampling times) were stored at the constant temperature of 5 °C, volatile and sensorial analysis were

carried out at the initial time and 1, 2, 3, 6 and 8 storage days . The effect of the packaging on sensorial quality and volatiles was compared with a control in air. In a second experiment, non-isothermal conditions in which 2 periods of 24 hours of temperature abuse (13 °C) were applied on day 2 and 6 of storage of 9 days at 5 °C, were compared to isothermal storage at 5°C. Nine bags were stored under these conditions, assessing gas concentration, sensorial attributes and volatile profile on 3 replicate bags at 0, 2, 5 and 9 days of storage. The effect of the packaging was compared to samples stored in isothermal condition at 5°C as a control.

6.1.3.2 Packaging gas composition

O_2 and CO_2 concentrations inside the packages were monitored with a gas analyzer WITT Mapy 4.0 (Witten, Germany). Test probe of gas analyzer was inserted into each package through an adhesive rubber septum to prevent air leaking from the package. After determining the gas composition, packages were used for sensory and volatile analysis.

6.1.3.3 Sensorial analysis

The attributes “visual quality” and “off-odors” of all samples were evaluated on by a five member trained panel. The visual quality was subjectively scored on a 5 to 1 scale, where 5 = excellent (fresh and turgid appearance, bright and uniform green colour), 4 = good (slight loss of turgidity and fresh appearance), 3 = fair (noticeable loss of turgidity and possible slight loss of green colour), 2 = poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = very poor (severe yellowing

of leafy blades and wilting, possible appearance of decay). A score of 3 was considered as the limit of marketability. Off-odors were scored on a 5 to 1 scale, where 1 = no off-odors, 2 = slightly off-odors, 3 = moderate off-odors (limit of marketability), 4 = strong off-odors and 5 = very strong off-odors, sulfur compounds. A score of 3 was considered as the limit of off-odor acceptability; over this limit the product is considered not marketable.

6.1.3.4 Volatile extraction and headspace SPME GC-MS analysis

Volatile compounds were evaluated in triplicate in each day of storage. 30 g of rocket leaves were added with 0.6 g of $CaCl_2$ and 6 g of NaCl and homogenized in presence of 30 mL of distilled water for 6 min using a commercial blender. For a single analysis, 8 g of the mixture, added with 2 µL of internal standard solution (IS, 2-methyl-1-pentanol, 100 ppm), were introduced into a 20 mL capped solid-phase microextraction vial. The sample vial was transferred into a water bath for 20 min, at 40 °C; then, an 85 µm fibre carboxen/polydimethylsiloxane (CAR/PDMS) was exposed for 30 min to the vial headspace and introduced into the GC injector port for desorption at 250 °C, for 4 min, in the split injection mode (1:20).

An Agilent gas chromatograph model 6890 Series coupled to an Agilent 5975 C network mass selective detector was used. Analytes were separated on a DB-WAX capillary column (60 m x 250 µm x 0.25 µm) by applying the following temperature program: 40 °C for 4 min, 40-140 °C at 3 °C/min, with a final holding time of 10 min. Transfer line temperature was 280 °C. Mass detector conditions were: electronic impact mode at 70 eV; source temperature 230 °C; scanning rate

2.88 scan/s; mass scanning range m/z 30-400. The carrier gas was helium at 1.0 mL/min. The identification of volatile compounds was achieved by comparing the mass spectra with the data system library (NIST 02, p>80). When available, the detected volatile compounds were compared with pure compounds; in the case of erucin, the compound was identified on the basis of mass spectrum (Table 2), in accordance with the literature (Blažević et al., 2008; Vaughn et al., 2005).

6.1.3.5 Statistical analysis

Data of the sensory score, gas composition and volatile compounds represent the mean of three replicates for treatment (standard deviation is calculated). Linear regression among volatiles of the same group and principal component analysis (PCA) of volatile data was carried out using Statistica software (ver.7, StatSoft, Tulsa, OK, USA) to determine changes in the aroma compounds during storage.

6.1.4 Results and discussion

The main sensory parameters, together with the volatile profile of packaged rocket were monitored for eight day storage, and compared with a control stored in air. A medium permeability PP film was chosen as too low oxygen levels can cause an increase in sulphides production (Nielsen et al., 2008). After 8 days of storage O_2 level inside the package was less than 7% and CO_2 level was at around 9 % (data not shown).

6.1.4.1 Volatile profile and sensory analysis of packaged rocket during the isothermal storage

The detected volatile compounds, at day 0 and 8 of storage, together with the relevant odor descriptors, are summarized in Table 1. Twenty-five compounds were identified; they represent the major polar volatile compounds of the rocket paste as leaves were grinded. According to Jirovetz et al. (2002), no qualitative difference was found in the aroma profile between leaves and the corresponding paste sample, but a higher factor for the odor intensity in leave paste.

Volatile compounds reported at time zero, C0, depicted the flavor fingerprint of fresh rocket, as they were present in all samples analyzed early in their shelf life, when product quality was maximum and included sulphur, C6 and C5 compounds, and lipid derived compounds.

A group of isothiocyanate and thiocyanate derivatives has been found, including erucin, 3-butenyl isothiocyanate (3-Bu-ITC), methyl thiocyanate (Me-TC), n-pentyl and 4-methylpentyl (4-MP-ITC) isothiocyanates, which originated from myrosinase activity on glucosinolates following tissue disruption (Halkier et al., 2006). These compounds gives the typical flavor notes to the fresh rocket (Bennett et al., 2006; Bennett et al., 2002; Jirovetz et al. 2002; Pasini et al., 2011) and have been reported to be important under healthy point of view (Yehuda et al., 2009).

C6 and C5 compounds (esters, alcohols, aldehydes and ketones) are generated via lipoxygenase pathway from fatty acids through a cascade of biochemical reactions. These compounds are responsible for the green-leaf odour (Jirovetz et al. 2002; Sigma-Aldrich, 2001;) of the leaves and constitute a plant defense mechanism when

they are mechanically damaged (Hatanaka, 1999; Reineccius, 2006). In all samples, (E)-2-hexenal, was the most abundant C6 aldehyde; hexanal, also present but in much lower amount, derives from either lipoxygenase action or from chemical oxidation, and has been related with green-grassy and green-apple odours. Within C6 compounds, the rocket at time zero also showed (Z)-3-hexen-1-ol, which has been associated with fresh green taste, and (Z)-3-hexen-l-ol acetate, responsible for green sweet notes. C5 compounds included penten-3-one described with spicy, pungent, ethereal scents, penten-3-ol, reported to have pungent, green vegetable nuances, (Z)-2-pentenol, found to be correlated with fruity notes, and pentan-3-one, typically described with ethereal acetone notes. Other minor compounds have been reported as deriving from lipid autoxidation, such as C7, C8 aldehydes (aldehydic odor type), and 2-pentylfuran (Nawar, 1999). 6-methyl-5-hepten-2-one, imparting a herbal, green, oily and pungent aroma, has been recovered from several types of vegetables including apple, tomato, and watermelon. According to the literature, it is considered an oxidative by-product or a product obtained from the degradation of lycopene, α-farnesene, citral, or conjugated trienols (Whitaker et al., 2000; Wolken et al., 2000).

Storage time caused dimethyl sulfide accumulation, which is reported to be formed both from (+)-S-methyl-L-cysteine sulfoxide (Marks et al., 1992) and by subsequent degradation of some volatiles derived from glucosinolates (Jin et al., 1999). Finally, also acetaldehyde (ethereal notes) and ethanol have been monitored as indicators of anaerobic respiration, because they have been demonstrated to accumulate in response to low O_2 and/or high CO_2 treatment for controlled

atmosphere storage of broccoli florets (Hansen et al., 2001).

In order to visualize differences between MAP and control rocket during the storage, multivariate analysis (PCA) was carried out, considering at the first step all volatile compounds as variables. All of them resulted with a score major than 0.9; therefore, the occurrence of correlations between variables within the same family was investigated, in order to explore the possibility to use some compounds as representatives in PCA analysis thus minimizing data redundancy. Within each compound family, a good linear correlation was observed for hexanal, 1-penten-3-ol, heptanal and 4-methylpentyl-isothiocyanate, therefore these variables were selected as representative of C6, C5, other lipid-derived and glucosinolate-derived compounds, respectively. Linear correlation coefficients are reported in Table 2. Additionally, acetaldehyde and dimethyl sulfide were used in PCA as known compounds with off-flavor notes (Kim et al., 2005; Nielsen et al., 2008). Ethanol did not accumulate with time, likely due to the not excessive reductive conditions into the packages (Table 1).

Figure 1 shows the score and loadings plots of data; PC1 and PC2 accounted for 61.76% and 24.21%, respectively. The first principal component (PC1) discriminated degradation of rocket in the time (loss of freshness), with samples at t0 in the negative part of the axes. The control condition caused a faster departure from the initial point representing product freshness, likely due to the loss of some volatile compounds together with a faster generation of off-flavors, whereas MAP-stored samples showed a slower odor degradation. PC2 discriminated between control and MAP at 8 days of storage, as the oxygen reduction and the

accumulation of CO_2 contributed to the formation of C5 compounds, besides to dimethyl sulfide and acetaldehyde which were confirmed to be markers of product degradation. This result was in accordance with Krumbein et al. (2010) observations, during studies on the changes of photochemical activity including volatile levels in Broccoli due to a controlled supply of atmospheric carbon dioxide in greenhouse environment.

As for sensorial analysis a panel of trained people judged the product for the presence of off-odor and for visual quality. Not significant differences were detected between control and packaged samples, even though a slight increase of off-odors in the MAP samples was observed at 8 days of storage (Figure 2), which may be explained with the alteration of the initial balance of rocket flavor in MAP stored samples, whereas the air-stored samples showed a slight increase in color degradation and subsequently deterioration of "visual quality". All the panelists, at 8 days, declared that the two samples had different but not-well describable odor notes, although both were judged with similar scores.

6.1.4.2 Volatile profile of packaged rocket during the non-isothermal storage

Volatile fraction was monitored also in non-isothermal conditions, with temperature fluctuating between 5 and 13°C at day 2 and 6, simulating an improper management of the cold chain, in comparison with MAP-stored samples in isothermal condition (5°C). In Figure 3 is shown the gas evolution in the packages for the 2 storage conditions. In the bags stored in isothermal conditions, O_2 and CO_2 reached the equilibrium already after 3 days being about 10 and 7 kPa, respectively. In samples

stored in non isothermal conditions, O_2 and CO_2 levels showed higher variation following the 2 exposures to temperature abuses, reaching at 9 days of storage almost 0 kPa of O_2 and 12 kPa of CO_2.

PCA was then carried out in order to understand if any trace of temperature history was left on volatile profile. Score and variable plots are reported in Figure 4; PC1 and PC2 accounted for 35.1% and 28.1%, respectively. It is worth noting that, even under fluctuating temperature conditions, off-flavors (dimethyl sulfide and acetaldehyde) can be used as markers of an improper temperature management as their levels raise with increasing temperature on day 2 and 6. Volatile changes due to the cold chain interruption were retained even after return to the cold storage, as suggested by the separation of samples stored in non-isothermal condition (marked with t values) from the control (marked with C values). Differently, production of C5 compounds did not seem to be linked to temperature changes. Sensory analysis detected the presence of off-odors for samples stored in non-isothermal condition at the day 6, when samples received a mean score of 2.3, whereas at the day 8 the score given to off-odor was about 4, suggesting that the faster deterioration of sample subjected to fluctuating temperature was also perceived by panelists (data not shown).

6.1.5 Conclusion

In conclusion results of this work provided useful information on changes of volatile profile of packaged rocket salad. Storage in MAP had a beneficial effect on the quality of rocket leaves preserving the freshness of the produce in terms of

visual quality and retention of the typical odor. However at the last days of storage an increased production of dimethyl sulfide and acetaldehyde was observed, which could respectively confer their typical sulfurous and ethereal odor to the produce. Moreover, the important role of temperature during storage is confirmed by results of the eperiment in non-isothermal conditions. Temperature fluctuation induced changes in the volatile profile compared to rocket stored in isothermal condition which persisted even when the cold chain was restored; the production of dimethyl sulfide and acetaldehyde gave a greatest contribute to the perception of an altered odor inside the package. These results suggest that these compounds can be effective markers to track temperature fluctuation in the product thermal history and these information can be useful for the development of a volatile sensor to early detect the quality and the shelf-life of rocket leaves.

6.1.6 References

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Table 1. Volatile compounds detected in control and MAP-stored wild rocket, at t_0 and t_8. (tr, retention time)

Compound	t_r	t_0	t_8 ctrl	t_8 MAP	odor descriptor
Anaerobic respiration compounds					
acetaldehyde	5.13	0.022±0.012	0.213±0.011	0.21±0.06	ethereal, pungent
ethanol	9.50	0.021±0.006	0.051±0.017	0.034±0.004	alcoholic, ethereal
C6 and C5 compounds					
pentan-3-one	10.84	0.06±0.03	0.31±0.08	0.17±0.06	ethereal
1-penten-3-one	12.49	0.40±0.05	1.1±0.2	2.1±1.2	spicy
hexanal	15.12	1.22±0.13	0.73±0.06	1.0±0.4	fatty, green, grassy
1-penten-3-ol	18.81	0.7±0. 2	3.0±1.0	2.3±0.5	pungent, green
(E)-2-hexenal	21.58	38±2	37±5	41±15	almond-like, green
(Z)-3-hexen-1-ol acetate	26.15	3.3±1.7	1.47±0.13	0.7±0.2	green sweet
(Z)-2-penten-1-ol	26.38	0.57±0.18	2.5±0.7	1.7±0.5	fruity, green
(Z)-3-hexen-1-ol	29.31	8±2	16±3	9±3	fresh, green grass-like
Lipid derivative compounds					
heptanal	19.93	0.075±0.032	0.02±0.02	0.004±0.004	aldehydic
2-pentylfuran	22.08	0.055±0.012	0.017±0.002	0.001±0.001	green, bean-like
6-methyl-2-heptanone	22.45	0.05±0.02	0.018±0.005	0.001±0.001	camphoreous
Octanal	24.84	0.04±0.03	0.018±0.003	0.001±0.001	aldehydic, waxy
(E)-2-heptenal	26.53	0.053±0.003	0.03±0.02	0.001±0.001	green
6-methyl-5-hepten-2-one	27.11	0.06±0.02	0.03±0.02	0.001±0.001	citrus
(E,E)-2,4-heptadienal	32.79	0.43±0.04	0.54±0.09	0.47±0.14	fatty
3,5-octadien-2-one	35.15	0.073±0.002	0.069±0.008	0.022±0.022	fatty
sulphur compounds					
dimethyl sulfide	5.58	0.009±0.010	0.18±0.04	0.09±0.03	sulfurous
methyl thiocyanate	24.03	0.21±0.12	0.65±0.12	0.41±0.11	sulfury onion
4-methylthiobutyl isothiocyanate[a]	28.58	42±2	30±10	36±17	cabbage
3-butenyl isothiocyanate	32.38	0.19±0.07	0.24±0.02	0.16±0.04	pungent
n-pentyl isothiocyanate	33.73	0.9±0.2	0.68±0.10	0.55±0.17	green
4-methylpentyl isothiocyanate	35.98	1.9±0.5	1.4±0.3	0.9±0.3	pungent, horseradish

[c]Compound identified by mass spectra comparison with literature values (Vaughn et Berhow, 2005; Blazevic et Mastelic, 2008). MS spectral data, m/z (relative intensity): 161 (M+, 20%), 146 (10%), 115 (75%), 85 (45%), 72 (28%), 61 (100%)

Odor descriptors using published data (Jirovetz et al. 2002 and references therein; Sigma-Aldrich, 2001)

Table 2. Linear correlation coefficients between heptanal and 2-pentylfuran, 6-ME-2-heptanone, (E)-2-heptanal, 6-ME-5-hepten-2-one, (E,E)-2,4-heptadienal, 3,5-octadien-2-one; between (Z)-3-hexenol acetate and hexanal, 2-hexenal, (Z)-3-hexenol; between (Z)-2-pentenol and 3-pentanone, 1-penten-3-one, 1-penten-3-ol; between 4-MP-ITC and n-pentyl ITC, 3-butenyl ITC, ME-ITC

	Linear correlations (R)						
Lipid derived compounds	2-pentylfuran	6-ME-2-heptanone	octanal	(E)-2-heptanal	6-ME-5-hepten-2-one	(E,E)-2,4-heptadienal	3,5-octadien-2-one
heptanal	0.60	0.93	0.70	0.89	0.83	0.59	0.76
C6 compounds	hexanal	2-hexenal	(Z)-3-hexenol				
(Z)-3-hexenol acetate	0.77	0.80	0.73				
C5 compounds	3-pentanone	1-penten-3-one	1-penten-3-ol				
(Z)-2-pentenol	0.59	0.61	0.90				
thiocyanic compounds	n-pentyl ITC	3-butenyl ITC	ME-ITC				
4-MP-ITC	0.99	0.93	0.62				

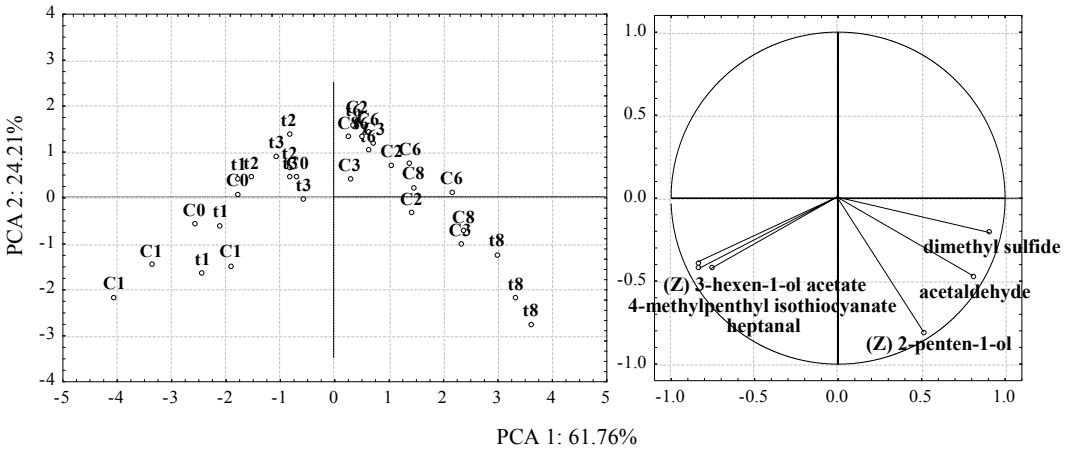

Figure 1. PCA analysis on the volatile compounds of the rocket stored in isothermal condition respectively in MAP (t values) and in air (C values). Mean values of 3 replicates (the numbers refer to the days of storage).

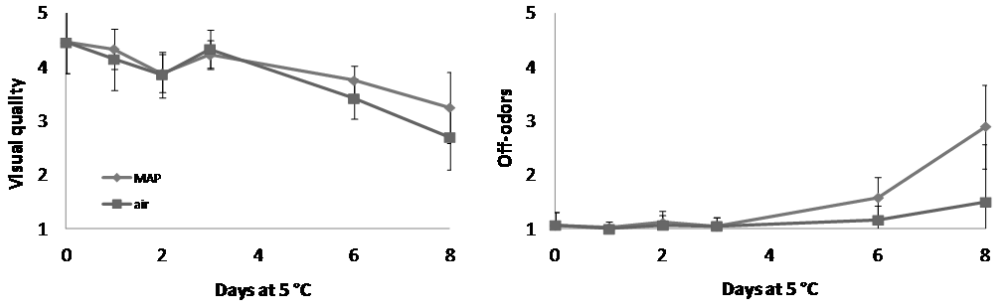

Figure 2. Sensorial analysis: (A) visual quality evaluation during the rocket storage; (B) development of off-odors during the rocket storage. Mean values of 5 evaluations ± standard deviation.

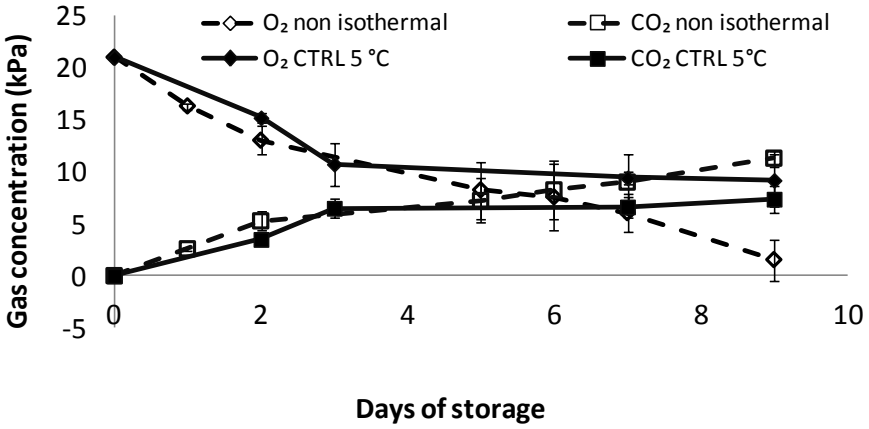

Figure 3. Gas evolution inside the rocket packages during the storage in isothermal (CTRL) and non isothermal conditions. Mean values of 3 replicates ± standard deviation.

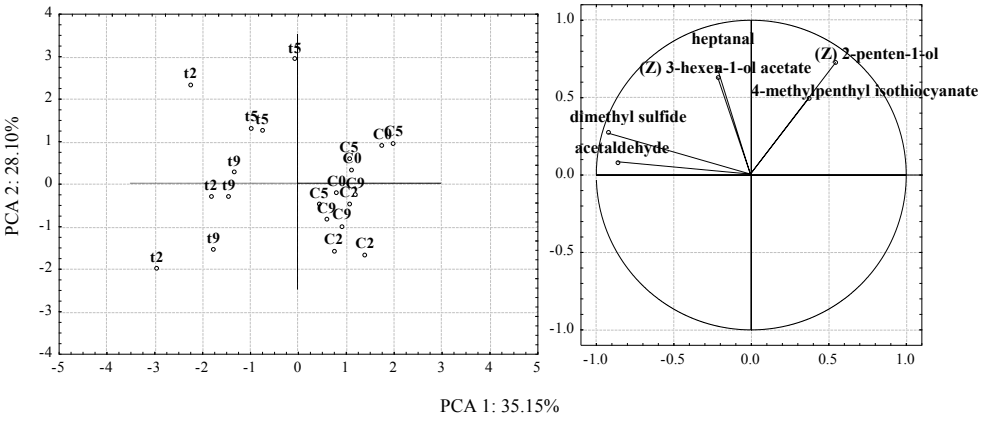

Figure 4. PCA analysis on the volatile compounds of the rocket stored in MAP for 9 days in non-isothermal condition (t values) and in isothermal condition at 5°C (C values) as control. Mean values of 3 replicates (the numbers refer to the days of storage).

7.1 EFFECT OF GAS COMPOSITION AND TEMPERATURE ON VOLATILE PROFILE ON ROCKET LEAVES PACKEGED IN MODIFIED ATMOSPHERES

7.1.1 Abstract

The aim of the this study was to investigate the effect of gas composition and temperature on volatiles profile of fresh rocket. Rocket leaves were packed and stored for 10 days at 0°, 5°C and 8 days at 15°C in order to evaluate the effect of temperature on aroma profile; moreover, storage in MAP at 5°C was also compared to rocket leaves stored in air as a control to evaluate the effect of gas composition on volatiles. Volatiles were extracted using solid-phase microextraction (SPME) directly in the package headspace and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Sensorial analysis were also carried out. Nineteen volatile were detected and grouped in three classes: sulphur compounds, including also isothiocyanates; compounds deriving from lipid metabolism and terpenes. Terpenes of MAP-stored rocket showed similar trends compared to the air-stored rocket suggesting that these typical odor compounds were not affected by gas composition; some lipid derivatives and sulphur compounds, known to be responsible of off-odors perception, were produced as a consequence of tissues degradation depending by temperature and gas composition, from the sixth day of storage in samples stored in MAP at 5°C, and three days in rocket stored in MAP at 15°C. The aroma was best preserved at 0°C, in which any degradation process was observed after 10 days of storage. Results indicated that storing fresh rocket leaves

in MAP at 0°C preserved aroma profile and that the gas composition and especially the temperature were important factors to take into account for a correct management of packaging conditions.

7.1.2 Introduction

Wild rocket (*Diplotaxis tenuifolia*) is one of the most popular leafy green eaten alone or in a mixture of salad in Europe. It is most appreciated for its sulphurous odour and characteristic bitter and pungent taste due to the presence of glucosinolates, typical compounds of *Brassicacea* family (Blažević et al., 2008). Rocket is available on the market as a raw produce or washed and packed in trays using polypropylene (PP) film to modify the gas composition inside the package (Løkke et al., 2012). Modified atmosphere is a common technique used to preserve the quality of fresh-cut produces; the management of the gas composition through the choice of appropriate gas levels inside the package could prolong the shelf-life and preserve the freshness of the produce (Sandhya, 2010). Martìnez-Sànchez et al. (2006) found that atmosphere enriched with 5 kPa O_2 and 10 kPa CO_2 was effective in preserving a good appearance of the leaves if compared to the storage in air, whereas production of undesirable odor in fresh-cut produce have been associated to high CO_2 levels inside the package (Nielsen et al., 2008). Koukounaras et al., 2007 reported a storage of 16 days for rocket leaves stored at 0 °C, increasing the temperature at 5 °C they observed a slight quality deterioration with a shelf-life of 13 days and found a rapid leaves deterioration using high temperatures, as 10 °C, with a shelf-life of 8 days. Temperature also affected the development of off-odors

in packed rocket increasing tthe accumulation of CO_2 and the O_2 consumption. The characteristic pungent flavour of fresh rocket has been described to be related to glucosinolates and their breakdown products isothiocyanates (Bennett et al., 2006; Bennett et al., 2002; D'Antuono et al., 2009; Pasini et al., 2011). They are formed when enzymatic reactions occur as a consequence of tissue damage causing the formation of a wide range of volatiles (Bones et al., 2006). The hydrolytic products have been described as pharmaceutically more active than their parent glucosinolate molecule (Faulkner et al., 1998). However, the characteristic aroma is not the result of one single odor impression but of an array of compounds; indeed, several compounds were found to be of essential importance (Jirovetz et al., 2002). Number and proportions of Eruca sativa volatiles also vary mainly in accordance with isolation methods including hydrodistillation-extraction by diethyl ether (Miyazawa et al., 2002), hydrodistillation in a Clevenger-type apparatus (Blažević et al., 2008), solid-phase microextraction (SPME) (Jirovetz et al., 2002). In particular, SPME has been demonstrated to be simple, rapid, solvent-free and effective for characterization purposes. In this work, volatiles sampled directly in the packaging headspace were determined by SPME-GC/MS in order to analyze changes in the volatiles profile related to temperature and gas composition during the storage, in modified atmosphere packaging, as perceived at the bag opening, without any manipulation of the leaves.

7.1.3 Materials and methods

7.1.3.1 Plant material and minimal processing

Fresh rocket leaves (*Diplotaxis tenuifolia*) were harvested in Salento (Apulia, Italy), washed in a free chlorine solution (0.01% v/v) before being drained, portioned into 50 g samples and packaged in PP bags (17.5x17.5 cm^2, OTR = 1800 $cm^3m^2d^{-1}$, WVTR = 6gm^2d^{-1}). Forty-five 'bags (3 replicates x 3 temperatures x 6 sampling times) were prepared and stored at 0, 5 and 15 °C. Additional 18 samples were stored at 5 ° in macroperforated bags as control in air. Volatile and sensorial analysis were carried out at time zero and and after 2, 3, 6, 7, 8 and 10 storage days for samples at 0°, 5°C while for samples kept at 15 °C sampling was interrupted at 8 days.

7.1.3.2 Packaging gas composition

O_2 and CO_2 concentrations inside the packages were monitored with a gas analyzer WITT Mapy 4.0 (Witten, Germany). Test probe of gas analyzer was inserted into each package through an adhesive rubber septum to prevent air leaking from the package. After determining the gas composition, packages were used for sensory and volatile analysis

7.1.3.3 Sensorial analysis

The attributes "visual quality" and "off-odors" of all samples were evaluated by a five member trained panel. The visual quality was subjectively scored on a 5 to 1 scale, where 5 = excellent (fresh and turgid appearance, bright and uniform green

colour), 4 = good (slight loss of turgidity and fresh appearance), 3 = fair (noticeable loss of turgidity and possible slight loss of green colour), 2 = poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = very poor (severe yellowing of leafy blades and wilting, possible appearance of decay). A score of 3 was considered as the limit of marketability. Off-odors and off-flavor were scored on a 5 to 1 scale, where 1 = no off-odors/off-flavors, 2 = slightly off-odors/off-flavors, 3 = moderate off-odors/off-flavors (limit of marketability), 4 = strong off-odors/ off-flavors and 5 = very strong off-odors/off-flavors, sulfur compounds and rotten cabbage taste. A score of 3 was considered as the limit of off-odor acceptability; over this limit the product was considered not markeTable. The odor was scored on a 3 to 1 scale (3 = typical/strong, already perceptible on intact leaves; 2 = typical, perceptible on broken leaves ; 1 = slight, the odor perception was limited to rubbed and manipulated leaves).

7.1.3.4 Volatile extraction and headspace SPME GC-MS analysis

Volatiles were collected in the bag headspace introducing the SPME fibre inside the package through a rubber septum when rocket leaves reached the temperature of 15°C. Samples stored in macroperforated bags used as control in air at 5 °C were transferred in no-perforated bags before sampling. A carboxen/polydimethylsiloxane (CAR/PDMS) fiber of 85 μm was exposed for 30 min to the bag headspace and introduced into the GC injector port at 250 °C, for a desorption time of 4 min, using the split injection mode (1:20). An Agilent gas chromatograph model 6890 Series coupled to an Agilent 5975 C network mass

selective detector was used. Analytes were separated on a HP-5ms capillary column (60 m x 250 μm x 0.25 μm) by applying the following temperature program: 40 °C for 4 min, up to 140 °C at 3 °C/min, with a final holding time of 10 min. Transfer line temperature was 280 °C. Mass detector conditions were: electronic impact mode at 70 eV; source temperature 230 °C; scanning rate 2.88 scan/s; mass scanning range m/z 30-400. The carrier gas was helium at 1.0 mL/min. The identification of volatile compounds was achieved by comparing the mass spectra with the data of a system library (NIST 02, p>80). In the case of isothiocyanates the compounds n-pentyl isothiocyanate and 4-methylpenthyl isothiocyanate were identified on the basis of mass spectrum, considering the ion fragments at 129 m/z and 143 m/z, respectively.

7.1.3.5 Statistical analysis

Data of the sensory score and volatile compounds represent the mean of three replicates for treatment (standard deviation is calculated). Linear regression among volatiles of the same group and principal component analysis (PCA) of volatile data was carried out using Statistica software (ver.7, StatSoft, Tulsa, OK, USA) to determine changes in the aroma compounds during storage. Data were also subjected to analysis of variance (one way anova); significant differences among treatments were evaluated by Tukey's honest significance difference test ($p<0.05$) using Statgraphics Centurion XVI software.

7.1.4 Results and discussion

Gas composition variations of rocket stored in MAP at 0, 5 and 15°C are described in Figure 1. In Table 1, volatiles found at time zero and after 8 days of storage, corresponding to the last storage day for rocket stored in MAP at 15°C, are reported, in order to appreciate the effect of temperature on volatiles profile. Packed rocket stored at 15°C, in fact, showed a high degradation rate resulting not markeTable already after 3 days of storage (data not shown). Nineteen volatiles were identified (Table 1) and grouped in three classes: sulphur compounds, including also isothiocyanates; lipid metabolism derivatives and terpenes. The choice of GC capillary column allowed a proper separation for several terpenes, which are secondary metabolites produced as a defense mechanism and known to give floreal notes to vegetables and fruits. Seven terpenes were identified (Table 1) but for only two of them a significant effect of the treatment was observed.

For β-myrcene, D-limonene, γ-terpinene and p-cymene, in fact, no difference due to the temperature could be observed even though the values at 0°C during storage were always higher than at 5°C and 15°C (data not shown). Lonchamp et al. 2009 reported that D-limonene could be considered as a marker for green color loss through the chlorophyll degradation in lettuce and could be an oxidation product of chlorophyll in the leafy green vegetable.

In Figures 2 and 3, the effects of storage temperature on β-pinene and 4-carene are reported. In addition, volatiles accumulation in control sample (stored in air) is reported in order to investigate the effect of gas composition on volatiles inside the package. These two compounds showed a different behavior from the other

terpenes; they were best preserved during the storage in MAP at 0°C. β-pinene was sensibly higher at 0°C than at the other temperatures up to 8 days of storage; no difference was detected between samples stored at 0 and at 5°C. As for the effect of gas composition, air-stored rocket leaves showed a major retention of β-pinene compared to leaves in MAP conditions; differences were found from the 6th storage day when gas composition inside the package was of 0.7±0.4 kPa O_2 and 24.2±1.9 kPa CO_2, suggesting that the establishing of anaerobic conditions could cause the degradation of this compound (Figure 1). Gas composition did not affect the 4-carene content (Figure 3) as obtained by one-way ANOVA at each storage day, although higher values were always observed for samples stored in MAP compared to rocket in air. As for temperature a higher retention was observed with lowering of temperature (Figure 3).

Most of the volatile compounds belonging to lipid derivative group showed an increase with the increasing of temperature when compared at 8 days of storage (Table 1). 2-methyl furan (Figure 4), 2-ethyl furan and 2-pentyl furan increased significantly during the 15°C-MAP storage, due to an accelerated membrane degradation caused by the high temperature. In the case of 2-methyl furan, peak area increased of about ten times when rocket was stored at 15°C, compared with the storage at lower temperatures. For 5°C-MAP stored samples, a significant increase was observed after 10 days, if compared to samples stored in air. The production of this compound in MAP-rocket suggested that gas composition affected the tissue degradation, which began in the last days of storage at 5°C in MAP, about 4 days later than for storage at 15°C. Nonetheless the effect of the

temperature was much more evident than the effect of the gas, since the content of 2-methyl furan in 5°C-MAP rocket at after 10 days was comparable to the value at time zero.

Nonanal (Figure 5) which also derives by fatty acid catabolism, decreased during storage without clear differences among temperatures for samples stored in MAP. On the other side, for control samples an increase was observed, resulting significantly higher than for samples stored in MAP starting from 3 days of storage. These results suggested that the presence of oxygen enhanced the lipid oxidation and that MAP storage could delay the fatty acid metabolism of rocket leaves even at high temperature as 15°C.

Also for sulphur compounds, a higher amount was found at 15°C compared to 0 and 5°C (Table 1). Particularly, in samples stored in MAP at 15°C the increase of isothiocyanates, starting from the third day of storage was observed. The trend for n-pentyl isothiocyanate, one of the volatiles responsible of the typical odor of rocket, is reported in Figure 6 as an example. A sharp increase of its content was observed at 3 days of storage for samples stored at 15°C (from a peak area of about $15x10^6$) and a considerable increase at 8th day of storage for samples stored at 5°C (final peak area of about $5x10^6$). Their formation, as for lipid derived compounds, could be due to membrane degradation processes and the consequent advance in enzymatic reactions, known to generate them (Halkier et al, 2006; Cavaiuolo et al, 2014). Moreover, significant differences were found comparing samples stored in MAP and in air, in fact degradation reactions are delayed in air resulting in a lower content than in rocket stored in MAP. For samples stored at 15°C, the gas

composition was of 0.92±0.97 kPa O_2 and 26.00±0.99 kPa CO_2 already after three days of storage (Figure 1), so, we can suppose that the established anaerobic condition could increase the degradation rate of cells structures and that temperature plays a key role in accelerating the degradation reactions.

A similar trend was found for dimethyl sulfide (Figure 7) and dimethyl disulfide (Figure 8) production; differences are detected between samples stored at 15°C compared to the other temperatures at 8 days of storage; the production of these volatiles strongly depended on temperature; in fact the amount in rocket stored at 15°C was very high compared with samples stored at 0°C and 5°C (Table 1, Figure 7-8). Dimethyl disulfide is also higher in rocket stored in air then rocket stored in MAP starting from the sixth days of storage; the presence of air could have accelerated the oxidation rate of sulfurous amino acid. Dimethyl disulfide content started to increase from the third day in rocket stored in MAP at 15°C, while its production in MAP conditions at 5°C began to be detected from the sixth days of storage. Dimethyl sulfide is reported to be formed both from (+)-S-methyl-L-cysteine sulfoxide (Marks et al., 1992) and by subsequent degradation of some volatiles derived from glucosinolates (Jin et al., 1999). Broccoli seedling stored in anaerobic conditions produced dimethyl sulphide and dimethyl disulfide through the degradation of cysteine containing protein (Derbali et al., 1998). Tetrahydrothiophene and thiophene are typical compounds of rocket (Jirovetz et al., 2002, Miyazawa et al., 2002) with alliaceous, sulfurous notes of cabbage-like; thiophene amount is maintained during the storage time and best preserved in MAP treatments at 0°C and 5°C whereas tetrahydrothiophene content showed differences

between samples stored at 0°C, 5°C and 15°C, in which an increased content was detected from the third day of storage. In order to visualize differences between the considered temperatures on MAP and control in air during the storage, multivariate analysis (PCA) was also carried out. Figure 9 shows the score and loadings plots of data; PC1 and PC2 accounted for 40.49% and 18.78%, respectively. The first principal component (PC1) discriminated degradation of rocket/production of off-odors related to temperature, with samples at 0, 5°C and in air, grouped in positive part of the axis, including also initial time (t0) and samples stored at 15°C in the first three days of storage. Samples stored at 15°C starting from 6 days were located in the negative part of the first principal component due to the development of off-odors associated to the production of sulphur compounds. Terpenes compounds were better preserved for samples stored in air and in MAP at 5°C. The second principal component (PC2) discriminated between control and MAP storage at 5°C. A development of off-odors in the samples stored in MAP at 10 days of storage could be caused, also in this case, by the production of sulphur compounds. All these results found a correspondence with the sensorial perception of the panelists who perceived off-odors starting from six days of storage, reaching the limit of acceptability at 7 days (data not shown). Leaves stored at 15°C produced off-flavors and off-odors already after the third day of storage, while a slight increase of off-flavor was observed for samples stored at 0°C (data not shown).

7.1.5 Conclusion

Temperature played an important role in enhancing the degradation rate of the rocket with the development of off-odors at higher temperatures. Volatiles responsible of off-odors as lipid derivatives and sulphurous compounds dramatically increased at 15°C . The typical aroma was best preserved at 0°C, in which no degradation process was observed during 10 days of storage. The effect of the MAP conditions compared to the control in air, was much lower than the effect of the temperature and was different depeding on the considered compounds. Storage in air helped to preseve beta-Pinene, a typical terpene characterisc of rocket aroma and delayed the formation of isothiocianate compounds. On the other side the presence of air enhanced the lipid oxidation in the case of nonanal. These volatile changes affected the panelist evaluation which perceived the presence of off-flavors starting from 6 days in MAP stored samples which were considered not marketable after 7 days, suggesting that formation of dimethyl sulfide and dimethyl disulfide may be the cause of consumer odor rejection. These results suggest that packaging design and control of the temperature during the whole product chain are crucial factors to preserve the aroma of rocket leaves.

7.1.6 References

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Table 1. Effect of MAP storage at 0°, 5° and 15°C on volatile composition during the fresh-rocket storage. Values of peak area are divided for a factor of 10^6; different letters after values of peak area mean significant differences for ($P<0.05$).

volatile compounds	rt	T0	0° MAP T8	5° MAP T8	15° MAP T8	odor descriptor
sulphur compounds						
dimethyl sulfide	3.69	0.54±0.03	0.53±0.03 b	10.96±2.20 b	574.12±181.37 a	sulfurous
dimethyl disulfide	8	0.86±0.01	0.74±0.06 b	2.65±1.67 b	5443.95±3061.44 a	sulfurous
thiophene	5.78	34.73±2.01	23.61±1.63	16.97±6.93	8.94±6.38	alliaceous, garlic
tetrahydrothiophene	10.44	0.75±0.06	0.71±0.01 b	0.74±0.03 b	475.06±171.91 a	sulfurous, cabbage-like
n-pentyl isothiocyanate	24.91	0.237±0.003	0.24±0.03 b	0.27±0.01 b	8.47±0.33 a	green
4-methylpentyl isothiocyanate	27.9	0.36±0.01	0.32±0.01 b	0.36±0.04 b	42.97±3.77 a	pungent, horseradish
Lipid derivative compounds						
2-methyl furan	4.68	40.29±11.49	3.38±1.14 b	7.77±3.40 b	482.05±94.48 a	ethereal, acetone, chocolate
2-ethyl furan	6.59	0.61±0.10	0.54±0.04 b	0.48±0.06 b	1159.76±256.66 a	chemical
2-pentyl furan	19.54	0.96±0.05	0.96±0.04 b	0.92±0.02 b	20.77±6.50 a	green, bean-like
(E,E) 2,4-heptadienal	20.5	0.44±0.17	0.57±0.02 b	0.57±0.03 b	7.05±4.26 a	fatty
nonanal	25.14	19.42±11.82	6.29±2.19	7.30±1.74	6.55±2.42	aldehydic
decanal	29.91	8.65±3.37	3.17±0.42	3.61±0.30	3.95±1.11	aldehydic
Terpenes compounds						
α-Pinene	16.58	7.30±1.00	2.55±0.76	3.60±1.42	5.74±4.42	herbal
β-Pinene	18.76	8.68±0.55	7.79±0.91 a	0.52±0.07 b	0.92±0.84 b	herbal
β-myrcene	19.52	0.70±0.10	1.79±0.29	1.69±0.46	0.47±0.06	spicy
p-Cymene	21.19	14.52±4.31	18.24±3.41	15.81±1.31	12.73±5.07	fresh, citrus, terpenoic
D-limonene	21.4	125.71±29.27	60.19±27.79	86.10±47.62	421.09±417.15	citrus, sweet, lemon
γ-Terpinene	22.99	5.49±0.37	3.85±0.85	4.17±0.67	2.71±3.26	oily, terpenoic, lemon
4-Carene	24.38	14.58±3.85	27.69±7.78 a	11.81±3.73 b	1.52±0.93 b	sweet and pungent odour

Odor descriptors using published data (Jirovetz et al. 2002 and references therein; Sigma-Aldrich, 2001)

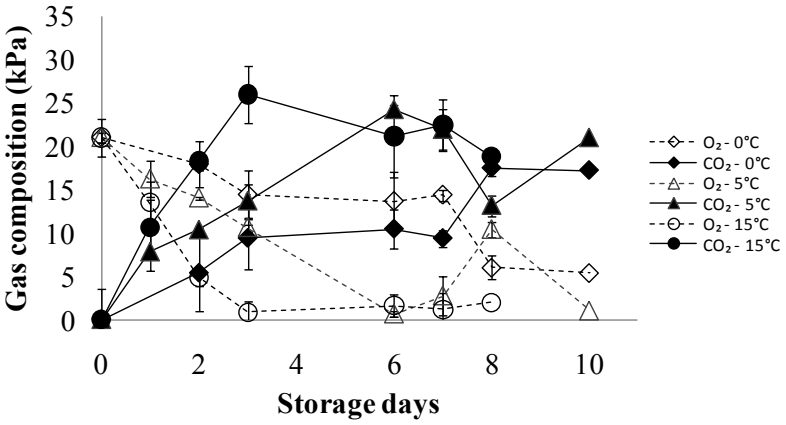

Figure 1. Gas composition variations during the rocket storage at 0, 5 and 15°C. Values are expressed as mean values of 3 replicates ± standard deviation.

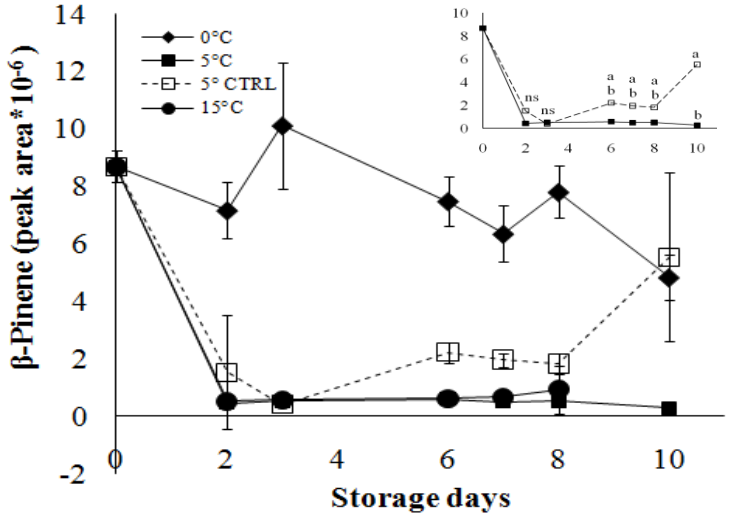

Figure 2. GC area peak relevant to β-pinene in rocket leaves stored in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP storage and 5°C-air storage. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

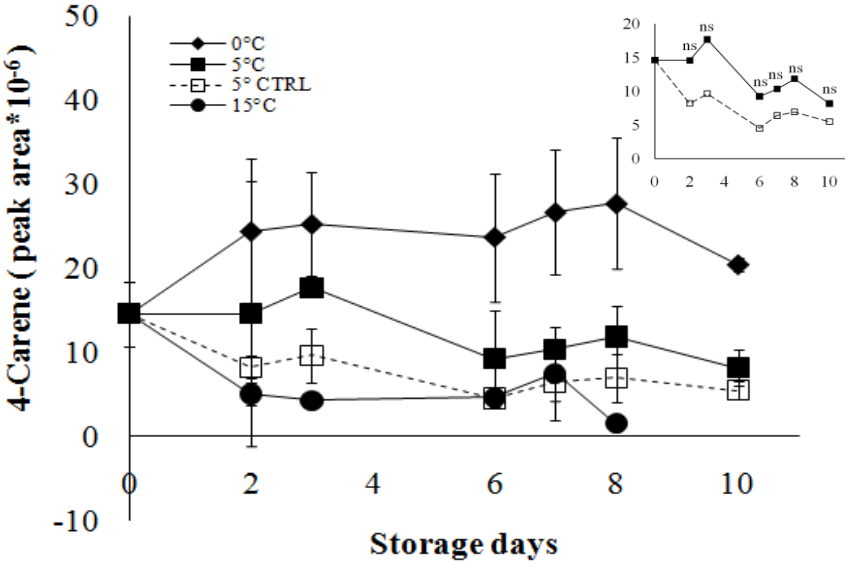

Figure 3. GC area peak relevant to 4-Carene in rocket leaves storage in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP storage and 5°C-air storage. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

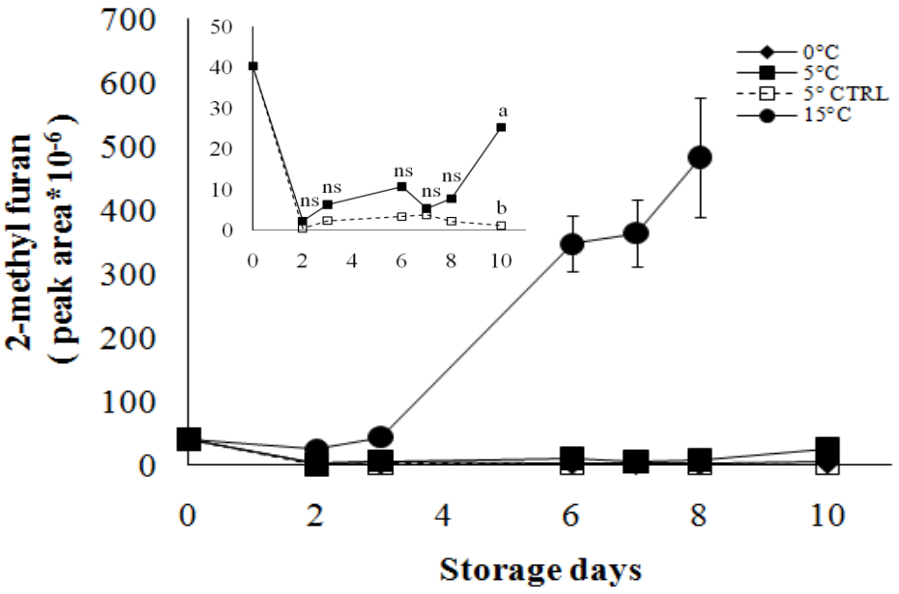

Figure 4. GC area peak relevant to 2-methyl furan in rocket leaves storage in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP and 5°C-air storage. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

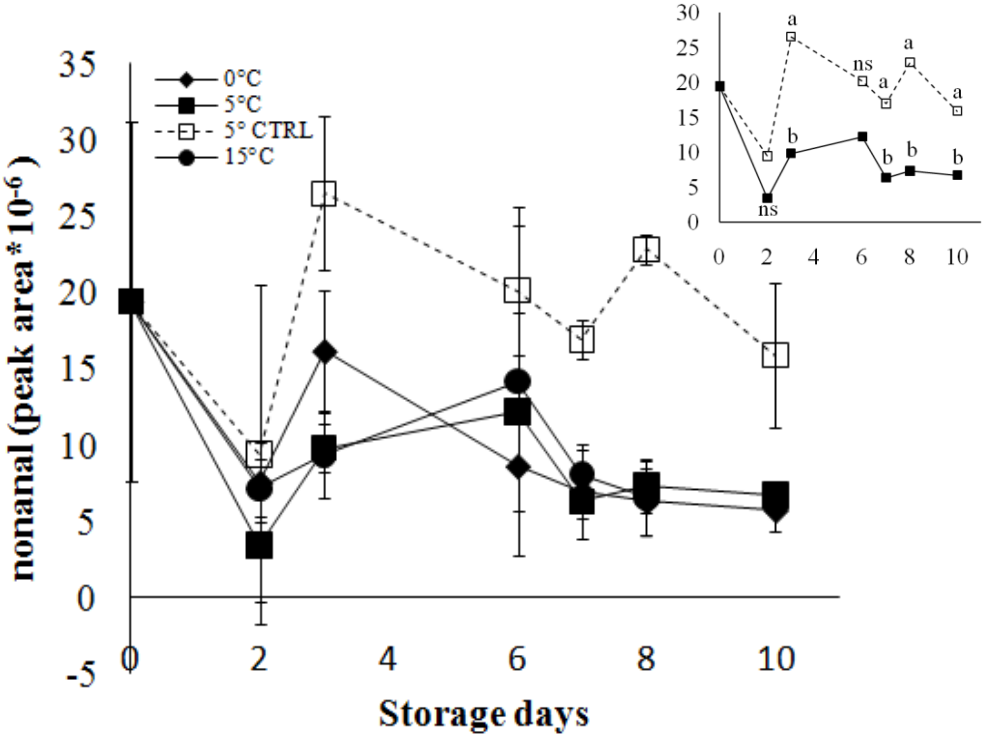

Figure 5. GC area peak relevant to nonanal in rocket leaves storage in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP and 5°C-air storages. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

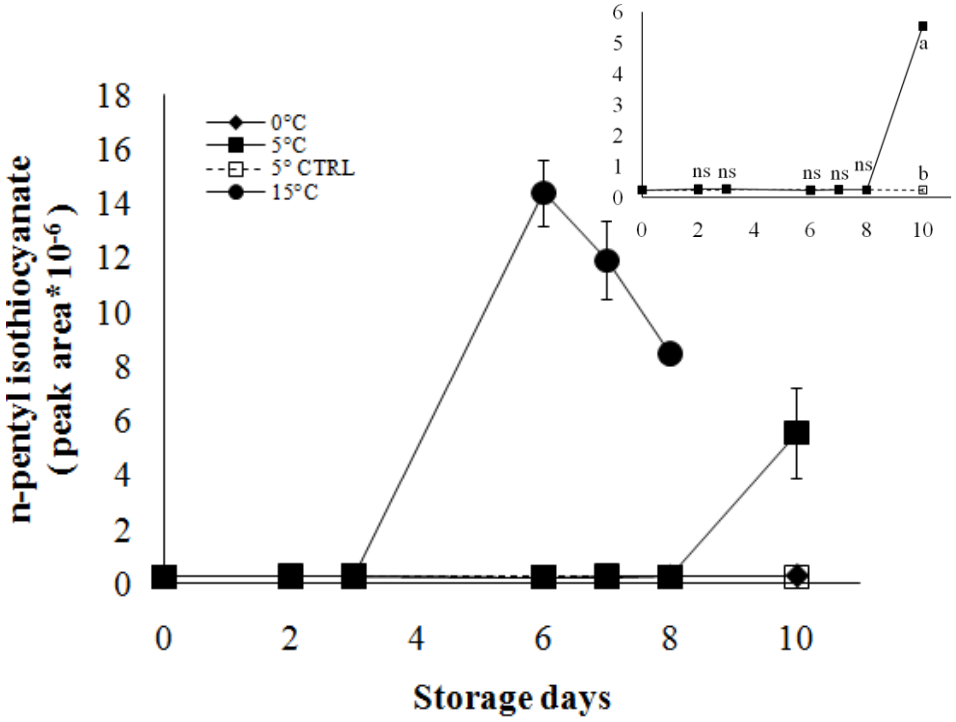

Figure 6. GC area peak relevant to n-pentyl isothiocyanate in rocket leaves stored in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP and 5°C-air storages. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

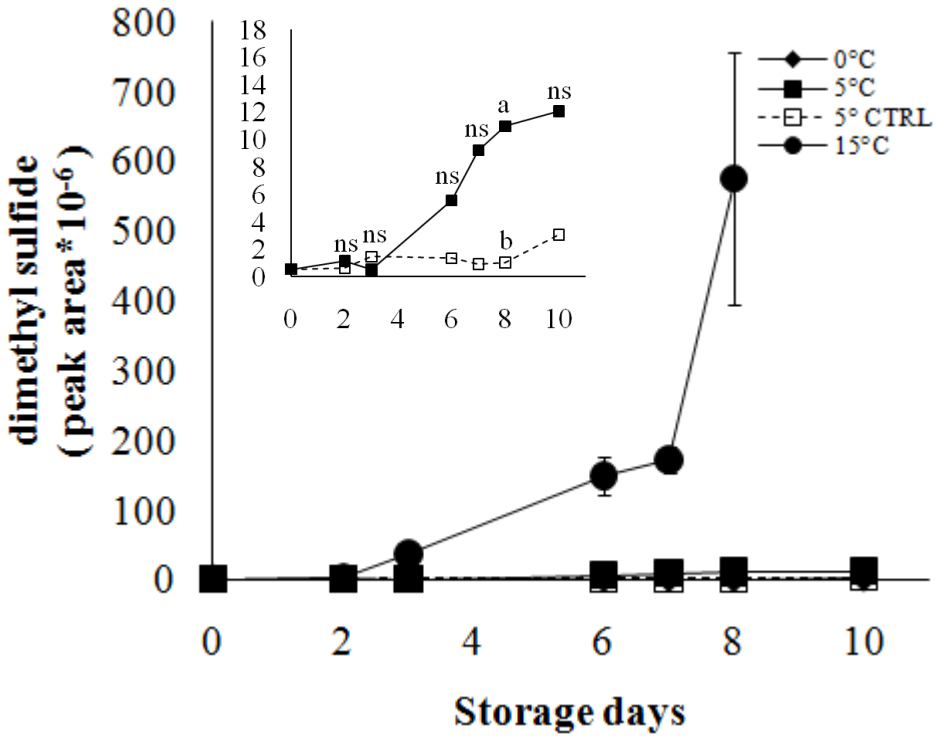

Figure 7. GC area peak relevant to dimethyl sulfide in rocket leaves storage in MAP at 0°C, 5°C and 15°C and comparison comparison between 5°C-MAP and 5°C-air storages. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

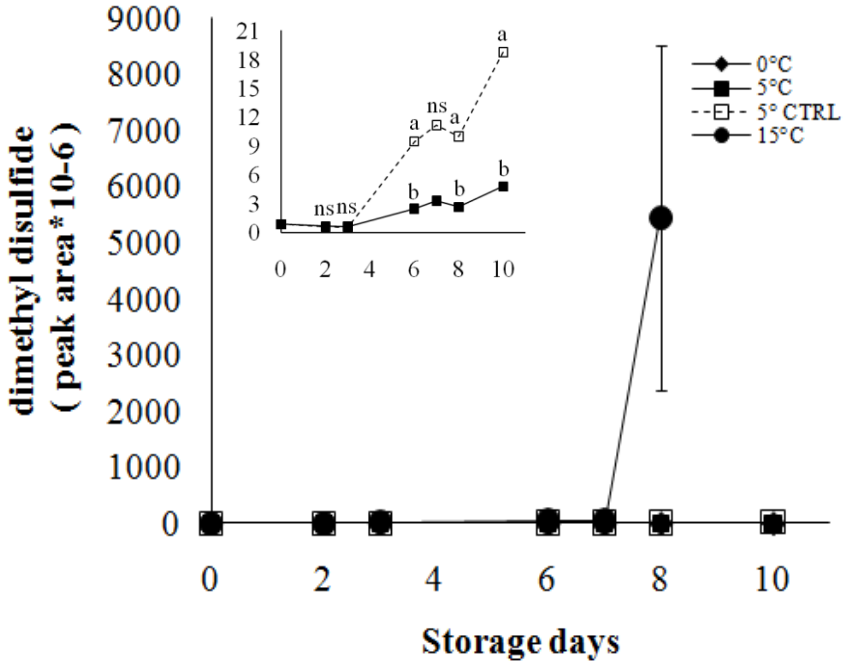

Figure 8. GC area peak relevant to dimethyl disulfide profile in rocket leaves storage in MAP at 0°C, 5°C and 15°C and comparison between 5°C-MAP and 5°C-air storages. Mean values of 3 replicates. Significant differences among treatments (ANOVA) were evaluated by Tukey's honest significance difference test ($p<0.05$)

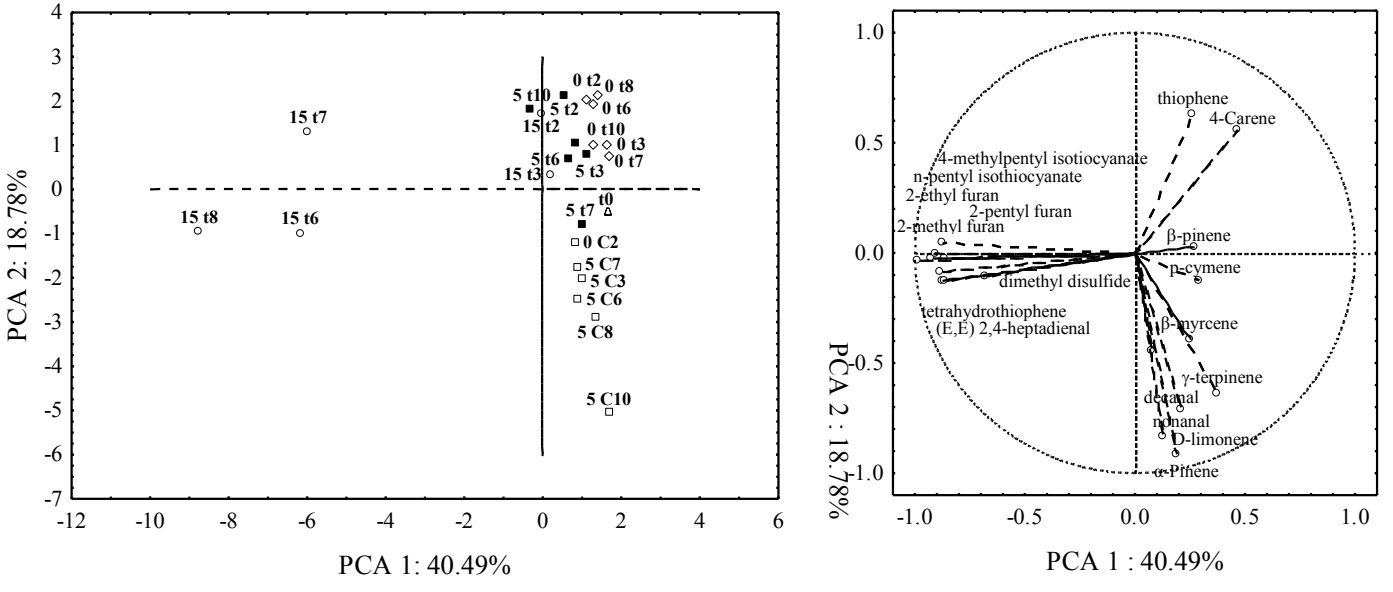

Figure 9. PCA analysis on the volatile compounds of the rocket stored in MAP at 0 (0 t_n), 5 (5 t_n), and 15°C (15 t_n), and in air at 5°C (5 C_n)

Mean values of 3 replicates (the numbers refer to the days of storage).

8.1 General conclusions

This work was part of the project "QUAFETY- *Comprehensive Approach to Enhance Quality and Safety of Ready to Eat Fresh Products*". The general aim was to improve the quality of ready-to eat rocket leaves (*Diplotaxis tenuifolia* L.) optimizing the storage conditions through a correct management of the temperature and the gas composition inside the packaging. This aim was reached by steps related to the results of different target experiments.

In the first trial the effect of temperature on the degradation kinetics of sensorial, physical and chemical attributes of rocket leaves was studied. Weibull model was compared to conventional first-order model to describe quality changes over time in function of the temperature. Weibull model showed an higher ability to fit experimental data compared to conventional first-order models, allowing to accurately describe shape and slope of the degradation curves. Obtained results also permitted to establish the most limiting factors for shelf-life of rocket leaves, which was therefore estimated in term of appearance score and ascorbic acid. This information should be used to optimize the logistic chain with the aim to increase the quality of fresh rocket delivered to the final consumer.

As second partial objective different concentrations of O_2 and CO_2 were independently tested to evaluate their potential beneficial or negative effects on qualitative attributes of fresh rocket during storage. Generally different oxygen concentrations did not had a high impact on quality of rocket leaves, resulting in a slightly high sensorial quality for the leaves stored under 3 and 6 kPa O_2, whereas O_2 concentrations below 0.5 kPa negatively affected the shelf-life of rocket leaves.

In conclusion even if the impact of the oxygen is not very critical, results of this experiment suggest to avoid concentrations as low as 0.5 kPa whereas the difference due to O_2 concentration from 3 to 20 were fairly noticeable. Whereas, the effects of high CO_2 were much more evident than those of low oxygen, indicating that the visual appearance was better preserved in presence of CO_2 compared to the storage in air. Despite this positive effect on appearance a strong production of off-odors was observed for rocket stored with 20 kPa CO_2, whereas level of 10 kPa CO_2 did not induced any off-odor development. As resulted from the volatile analysis the major contribute to the perception of altered odor for leaves stored with 20 kPa CO_2 could be due to the presence of 2-butanone and 2-pentanone. On the other side, the off-odors perceived during the storage in air could be associated to dimethyl sulfide and dimethyl disulfide content. Even if the impact of the oxygen is not very critical, results of this experiment suggest to avoid concentrations as low as 0.5 kPa whereas the difference due to O_2 concentration from 3 to 20 were fairly noticeable storing fresh-cut rocket leaves in 10 kPa CO_2-enriched atmosphere was the most beneficial atmosphere to preserve quality of rocket leaves and that higher concentrations should be avoided.

Third objective of this work was to evaluate the effect of the temperature on rocket leaves packaged in passive modified atmosphere, where gas composition is in turn affected by the temperature. Degradation kinetics of the quality attributes on fresh-cut rocket were modeled in isothermal and non isothermal conditions in order to improve the management of the logistic chain according to the product thermal history. In particular, the cumulative form of the Weibull equation and a log-logistic

model were, respectively, used to fit the experimental data over time and to study the temperature dependence of the degradation rates for several sensorial, chemical and physical attributes. Weibullian-logistic model was also able to predict the shelf life of fresh rocket leaves under isothermal and non-isothermal conditions; in particular, any temperature profile detected along the entire cold chain could be used to define the "best before" date reported on the packages.

Moreover, since MAP beneficial effects can be lost if anaerobic conditions develop inside the packaging due to an improper packaging management, volatile changes in MAP stored rocket leaves were analyzed in isothermal (5°C) and non isothermal conditions. Storage in MAP had a beneficial effect on the quality of rocket leaves preserving the freshness of the produce in terms of visual quality and retention of the typical odor. However at the last days of storage an increased production of dimethyl sulfide and acetaldehyde was observed, which could respectively confer the typical sulfurous and ethereal odor to the produce. Moreover, the important role of temperature during storage is confirmed by results of the experiment in non-isothermal conditions. Temperature fluctuation induced changes in the volatile profile compared to rocket stored in isothermal conditions which persisted even when the cold chain control was restored. The production of dimethyl sulfide and acetaldehyde gave a greatest contribute to the perception of an altered odor inside the package. These results suggest that these compounds can be effective markers to track temperature fluctuation in the product thermal history.

Finally a last experiment was aimed to monitor the volatiles accumulated in the headspace of the packages, without enzymatic reactions induced by leave

homogenization, to evaluate the effect of gas composition and temperature on the compounds responsible of the perceived odor after the opening of the package. To this aim, rocket leaves were packed and stored at 0, 5 and 15°C; additionally at 5 °C a control in air was also included to test the effect of gas composition on volatile profile. Temperature played an important role by accelerating the degradation rate of the rocket leaves inducing the development of off-odors. At 15 °C temperature lipid derivatives and sulphurous compounds were in fact produced in much higher quantity than at low temperatures. The typical aroma was best preserved at 0°C, in which no degradation process was observed in the last days of storage. The effect of the MAP conditions compared to the control in air, was much lower than the effect of the temperature and was different depending on the considered compound. The presence of air enhanced the lipid oxidation in the case of nonanal, but at the same time helped to preserve beta-Pinene, a typical terpene characteristic of rocket aroma and delayed the formation of isothiocianate compounds. These volatile changes may be the reason of the presence of off-flavors perceived by the panelists starting from 6 days in MAP stored samples which were considered not markeTable after 7 days. These results suggest that packaging design and control of the temperature during the whole product chain are crucial factors to preserve the aroma of rocket leaves.

Generally the results of this work of thesis increased the knowledge on degradation reactions occurring during storage of rocket leaves under different temperature and gas conditions. Several physical, sensorial and nutritional quality, including volatiles were taken into consideration at the same time. This allowed to define the

most critical attributes limiting the shelf-life and the critical factors to control during the processing chain. Moreover among volatiles some quality markers for temperature abuse and not optimal gas conditions were identified. The high accuracy of the curve fitting describing the dependence from the temperature of many quality attributes allowed an accurate shelf-life prediction. All these aspects would allow processors and distributors to improve the management of the critical control points and quality maintenance during the entire production chain. This is important for planning produce logistics with fully automated distribution steps and better manage stocks, according to thermal history and, possibly, to priorities of potential customers. At the same time consumers will beneficiated of the increased and standardized quality and of having high level of information on the fate of nutritional compounds.

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