UNIVERSITÀ DI FOGGIA
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Doctoral Thesis in
Management of Innovation in the Agricultural and Food System of the Mediterranean Region
- XXVIII cycle -

OPTIMIZATION OF CRITICAL ASPECTS FOR PROCESSING FENNEL (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) AS A FRESH-CUT PRODUCT

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***Doctorate Course in 'Management of Innovation in the Agricultural and Food System of the Mediterranean Region'*- XXVIII cycle –**

Doctoral thesis on **'Optimization of critical aspects for processing fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) as a fresh-cut product'**, discussed at the Università di Foggia, May 5, 2016

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Optimization of critical aspects for processing fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) as a fresh-cut product

Contents

Abstract

Fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) is one of the most popular vegetable in Italy that is commonly eaten raw either alone or mixed in a salad, or cooked as vegetable. Consumers mainly appreciates this crop for its organoleptic properties such as sweet-taste, aroma and aniseed-flavour but also for its crunchiness. However the high percentage of discarded plant waste, together with complex and time-consuming trimming operation, may discourage consumers, affecting their decision to buy. Consumers often have limited time to spent purchasing, storing, preparing and consuming food. In addition, due to growing health awareness, they are more concerned about the nutritional, sensorial and safety aspects of the food they eat. From these consideration, fresh-cut processing is very desirable for fennel, since it is still not available as a fresh-cut, high-convenience, ready-to-eat-product. As a fresh-cut product fennel would be extremely susceptible to browning of the cut surfaces, and this might limits its shelf-life. Therefore, in order to remove technological constraints that prevent its availability as a fresh-cut product, an integrated approach was used starting from the selection of maturity of raw material to processing and packaging in order to optimize the critical aspects for the production of fresh-cut fennel.

The maturity stage is a critical factor for the quality at harvest and after fresh-cut processing. The aim of the **first study** was to evaluate the effect of maturity on quality of fennel heads and on their browning susceptibility after cutting. Fennel heads (cv. Tiziano) were harvested at 7 stages of maturity over a period of 21 days, from HT1 (immature) to HT7 (over-mature), with HT3 as the usual commercial

maturity stage. Quality attributes were evaluated at harvest and after 4 days of storage at 5°C. Fennel heads reached the full size at maturity stage HT3. Maturity at harvest significantly affected respiration rate and quality attributes. Respiration rate increased from HT1 to HT4 and then remained almost constant until HT7. A gradual loss of green color occurred from HT1 to HT7, in both stems and sheaths. Sugars as well as organic acids reached highest values at HT3 and then decreased until the over-mature stage. Total phenol and vitamin C contents showed similar trends, with lower values at HT1 compared to HTs 2, 3 and 4, and then starting to decrease after HT5. Regardless of the HT, after 4 days at 5 °C in air fresh-cut fennels turned brown on the cut-surfaces of stems and on sheaths; however the lower total color variation was observed in samples harvested at HT2. Results showed that there is a very restricted range of time to harvest fennels in order to have a good quality fresh-cut product. Harvesting fennels heads at the commercial maturity stage ensures high nutritional values and good sugar content along with the highest process yield (since fennels reached the full size). However a slight anticipation of the harvest time could reduce the occurrence of browning in both stem and sheath cut-surfaces of fennel slices during post-cutting storage.

The objective of the **second study** was to use hyperspectral imaging to predict the internal content of different quality attributes such as soluble solids, individual sugars and organic acids, phenols, and antioxidant activity of fennel heads also in relation to different sheat layers and harvest times. Thirthytwo fennel heads were collected during 7 different harvests as described in the previous experiment. For each fennel 2 images of the perpendicular section (cut in the middle of the head)

were acquired with a Hyperspectral scanner by using 2 spectrographs in the VIS NIR(400 to 1000 nm) and in the NIR region (900-1700 nm). For prediction purposes, 5 leaves (including the stem) were individuated from the external to the internal part and grinded to get the tissue puree to be used for chemical extraction, obtaining a total of 160 samples. In the same way from hyperspectral images more regions of interest extracted for each corresponding leaf were averaged obtaining a total of 160 spectra. Reference content values were then used to build the Partial Least Square Regression (PLSR) for each of the 2 spectral datasets. Among the predicted parameters only phenols, total soluble solids, and antioxidant activity could be predicted with satisfactory accuracy whereas the other compounds were predicted with very low performances. For all these 3 parameters VIS-NIRS gave better results than NIR spectra, and this is probably because some information is retained in the color and also because the maximum absorbance value for the standards of the reference method. Moreover it is interesting to observe that soluble solids, phenolics and antioxidant activity increased from the external to the internal leaves, and that this variation can be observed on hyperspectral images by mapping the constituent concentrations. Classification based on time of harvest allowed to distinguish all classes with a non-error rate of 92.29% in calibration and 81.86% in cross validation. To improve classification performance, similar samples were merged in 4 new classes (harvest 1, harvest 2 and 3, harvest 4, and harvest 5, 6 and 7), resulting in a significant rise of non error rate. In conclusion results of this study show the potentiality of hyperspectral imaging in the VIS-NIR spectral range to

predict internal content of soluble solids, phenols and antioxidant activity and to classify fennel heads according to the harvest time.

The occurrence of browning is the main cause of quality loss and decrease of visual acceptance for fresh-cut fennel. In the **third study** the effectiveness of different antibrowning solutions on maintaining quality characteristics of fresh-cut fennel during storage at 5 °C were investigated. Results showed that dipping in solutions of citric acid, ascorbic acid, cysteine, did not result in substantial improvement of the appearance of fresh-cut fennels cut-surfaces compared to water control. Among all applied antibrowning solutions, dipping in 1% citric acid and 0.5% 4-hexylresorcinol produced a more severe browning than control, in both stem and sheath cut-surfaces. Dipping for 2 minute in 0.5% ethanol was effective in preserving visual quality of fresh-cut fennel stored in air for 6 days at 5 °C, significantly reducing the browning in both stem and sheath cut-surface during storage. In addition ethanol is a 'generally recognized as safe' (GRAS) product and did not negatively influence the aroma of fresh-cut fennel. Based on these considerations, the dipping in 0.5% ethanol for 2 min could be a useful pretreatment for extending the shelf-life of fresh-cut fennel.

The objective of the **fourth study** was to understand the effects of atmosphere modification on fresh-cut 'Apollo' fennel and to identify best suitable gas mixture to extend its shelf-life. Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Apollo*) were trimmed, sliced, dipped in EtOH 0.5% as antibrowning agents, and stored for 14 days at 5 °C in different controlled atmosphere (CA) conditions. Two different experiments were carried out. In the first experiment the

applied CA conditions were the following: 2 kPa O_2 in nitrogen, 20 kPa CO_2 in air, 2 kPa O_2 + 20 kPa CO_2 in nitrogen, and Air (as a control). Results showed that an atmosphere of air enriched with 20 kPa CO_2 was effective to preserve the appearance of fresh-cut fennel stored at 5 °C for 14 days, delaying the occurrence of browning on the cut surface of fennel slices. When the oxygen level was decreased to 2 kPa in the presence of 20 kPa CO_2, the effectiveness of CO_2 on controlling stem browning slightly decreased. Results from a wider range of atmosphere compositions tested in the second experiment to further clarify the effect of atmosphere modifications on total color variation of the cut surface substantially confirmed previous finding. When only O_2 concentration was lowered no control of browning was observed, with a similar loss of visual quality as detected in control samples stored in air. From a nutritional point of view no significant changes were observed in terms of antioxidant capacity, phenolic and ascorbic acid contents in relation to the applied CA conditions. On the other hand the microbiological quality was significant influenced by the presence of CO_2 as the growth of mesophilic bacteria was delayed; the lowering of oxygen seemed to be not effective on the inhibition of mesophilic population while it affected the count of yeasts, retarding their growth. Taking into account the overall changes of quality parameters of fresh-cut fennel over time, the model obtained using multivariate analysis confirmed that samples stored in air enriched with 20 kPa CO_2 showed the slowest degradation kinetic. However, it should be considered that the production of fermentative metabolites could occur in this storage condition. In addition, an atmosphere of 20 kPa CO_2 in air is not feasible to be obtained in modified

atmosphere packaging (MAP),since due to the film permeability, the CO_2 accumulation cannot be unrelated to oxygen consumption.

The aim of the **fifth study** was to design a modified atmosphere packaging (MAP) in bags in order to reach the optimal gas composition (2-5 kPa O_2 + 20 kPa CO_2), as resulted from previous studies. In a first experiment fennel heads were cut, dipped in EtOH 0.5% and kept in air or packed in polypropylene film (PP) without (NMP) or with one (MP1) or two (MP2) layers of microperforation, flushing an atmosphere of 5 kPa O_2 + 20 kPa CO_2 in nitrogen inside the bags. All samples were stored at 5 °C for 10 days, evaluating the gas changes over storage time. PP NMP and PP MP2 were discarded since rapid anoxic conditions (in PP NMP) and a too high gas exchanges (in PP MP2) occurred already after 24 h of storage, while in PP MP1 samples a steady state of about 12 kPa O_2 and 10 kPa CO_2 was rapidly reached. Despite not reaching the target gas concentrations PP MP1 resulted effective in reducing browning of the fennel cut-surfaces, to better maintain the nutritional values and to avoid the loss of weight compared to control in air. In a second experiment a passive MAP was used testing 2 different plastic material (PP MP1 and PP+PA MP1) in order to optimize packaging design. Better results were obtained with PP+PA MP1 that allowed to reach the desired gas concentration inside the bags. In a third experiment fennel heads cv. *Apollo* were processed as described above and samples of about 150 and 200 g were closed in PP MP1 and PP+PA MP1 respectively, flushing an initial atmosphere of 5 kPa O_2 + 20 kPa CO_2 in nitrogen inside the bags. CTRL samples of about 150 g were kept in air. Changes in gas composition in PP MP1 and PP+PA MP1 samples were monitored over time.

Samples were evaluated initially and after 3, 7 and 13 days of storage at 5 °C. Suitable gas composition (5 kPa O_2 + 20 kPa CO_2) were maintained in PP+PA MP1 samples over time, which proved to be effective in delaying browning in both stem and sheath cut-surfaces, and in controlling the mesophilic and psychrophilic growth as well as enterobacteriaceae contamination up to 7 days compared to CTRL. In terms of nutritional quality, a loss of vitamin C occurred in all treatments while no changes over time were observed for phenolic compounds, sugars and organic acids. Therefore, based on results of the present experiments, packaging 200-250 g (depending on the respiration rate) of fennel slices, dipped in ethanol 0.5%, in PP+PA MP1 bags (15 x 20 cm) with initial gas composition of 5 kPa O_2 and 20 kPa CO_2 is effective in maintaining a very good visual quality, without main nutritional losses. In addition shelf-life in all the tested conditions, was estimated applying the Multivariate accelerate shelf-life test (MASLT). Based on the model obtained, the shelf-life for stored fresh-cut fennels was 9.7, 12.2, and 24.2 days for air, PP MP1 and PP+PA MP1 conditions, respectively.

The results of this thesis increased the knowledge on some of the critical aspects for minimally processing fennel, providing important information to improve pre- and post-cutting handling in order to remove technological constrains for the production of a value-added, convenient, ready-to-eat fennel product.

Key words: fennel, fresh-cut, browning, MAP, MALST, Vis/NIR.

PART ONE: INTRODUCTION

1.1. FRESH-CUT AND READY TO USE FRUITS AND VEGETABLES

Consumers are more concerned about the nutritional, sensorial and safety aspects of the food they eat due to growing health awareness (Qadri et al., 2015). In particular in recent years the attention towards a healthy diet is considerably growing (Corbo et al., 2015) and, as results there is an increasing demand for healthy and nutritious products. The increase in consumption of healthy food is associated with an abundance of scientific evidence of a possible connection between diet and good health. The rising costs of health care coupled with a high incidence of obesity and diet-related disease have also led to increased public concern about health and nutrition (Benedict et al., 2015). Fruits and vegetables are the major dietary sources of nutrients of greater importance from the human nutritional point of view (Qadri et al., 2015). A significant amount of epidemiological evidence has demonstrated that the consumption of vegetables and fruits is beneficial to health (Boeing et al., 2012). The beneficial health effects of fruit and vegetables have been attributed to the presence of antioxidants that act as receptors of free radicals. Ascorbic acid and β-carotene are the antioxidants present in the greatest quantities in fruit and vegetables (Rico et al., 2007) but other antioxidant phytochemicals contained in fruits and vegetables may be equally important (Prior et al., 2000). In addition fresh fruits and vegetables are strongly recommended in the human diet as a source minerals and of dietary fiber (Boeing et al., 2012; Slavin et al., 2012). Organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), United States Department of Agriculture (USDA), and European Food Safety Authority (EFSA) recommended an increase of fruits

and vegetables consumption to decrease the risk of cardiovascular diseases and cancer (Allende et al., 2006). According to the World Health Organization (WHO), the average daily recommended intake of fruits and vegetables is more than 400 grams per capita (WHO, 2008). However, it is well-known that modern lifestyles and eating habits usually tend to a reduction of suitable intake of rich sources of antioxidant compounds, such as fruit and vegetables, being more emphasized in some parts of the population, especially children (Allende et al., 2006). For instance the last *Consumption Monitor* of European Fresh Produce Association (2012) shows that overall, 2011 experienced a slight 2.6% raise in the consumption pattern to 382 g/capita/day for fresh fruits and vegetables on average for the EU-27 (Freshfel, 2013). In addition the lifestyles of today's society are very different to that of 20 or even 10 years ago. The consumer profile is changing as an effect of a stressed lifestyle (Corbo et al., 2015). The many different activities that people nowadays need or want to combine, have become so complex, that people are increasingly looking for ways to save time, including into their home-kitchen (Daniels et al., 2015). The increased demand for convenience foods illustrates the impact of changing lifestyles on demand. Convenience-related quality is linked to more than just the time spent in the kitchen—it covers time and effort (mental and physical) spent purchasing, storing, preparing and consuming food (Buckley et al., 2007). As a response to consumers' demand for healthy, fresh-like and easy to prepare products, conjoint with consumer lifestyle changes, a wide variety of minimally processed fruits and vegetables has been developed (Ramos et al., 2013). According to the definition of the US Food and Drug Administration, fresh-cut

fruits and vegetables or fresh-cut produce are "fresh fruits and vegetables for human consumption that have been minimally processed and altered in form by peeling, slicing, chopping, shredding, coring, or trimming, with or without washing, prior to being packaged for use by the consumer or a retail establishment" (US FDA 2008). In Europe, fresh-cut products were introduced in France in the early 1980s by Florette Group. It was the first production unit of fresh-cut vegetables in Europe which subsequently started various activities to export to other country such as the United Kingdom, Italy, and Switzerland (Rojas-Graü et al., 2010). Since 1980s the market for fresh-cut fruits and vegetables in Europe has grown enormously and amounted to about 3 billion euro in 2014: the British market covers about 33% of the total sales of fresh-cut products, followed by Italy (26%), France (18%), Spain (12%) and Germany (11%) (VVA Brussels, 2015). The study underlines that consumers purchase fresh-cut products for saving time, because they are already washed and adequately portioned, and because there is a waste reduction. In addition consumers pay attention to healthy diet, try to reduce the consumption of meat (vegetarians, vegans, and "integralists") and search for local and '0-km' or "proximity" products (VVA Brussels, 2015). Recent data from the 'Monitor Ortofrutta' Observatory of Agroter (Monitor F&V 2015) which probed the orientation of Italian consumers about fruits and vegetables, shown that in 2015 the consumption for fresh-cut fruits and vegetables in Italy had an increase of +2.2% compared to biennium 2012-2014, and the market sales is about 1 billion euro. Concerning the features of the market in Italy, packaged salads appear to be the leader of fresh-cut products, in fact it holds about 75.2% of the total, followed by

rocket (9.5%), spinach (4.5%), carrots (2.6%), Swiss chards (1.3%), mushrooms (1.2%) and cabbages (0.7%) (Figure 1.1). The remain 4.8% is share by others fresh-cut vegetables and fruits. In particular fresh and fresh-cut fruits shared only in 2.7% of the total fresh and fresh-cut market (Monitor F&V 2015).

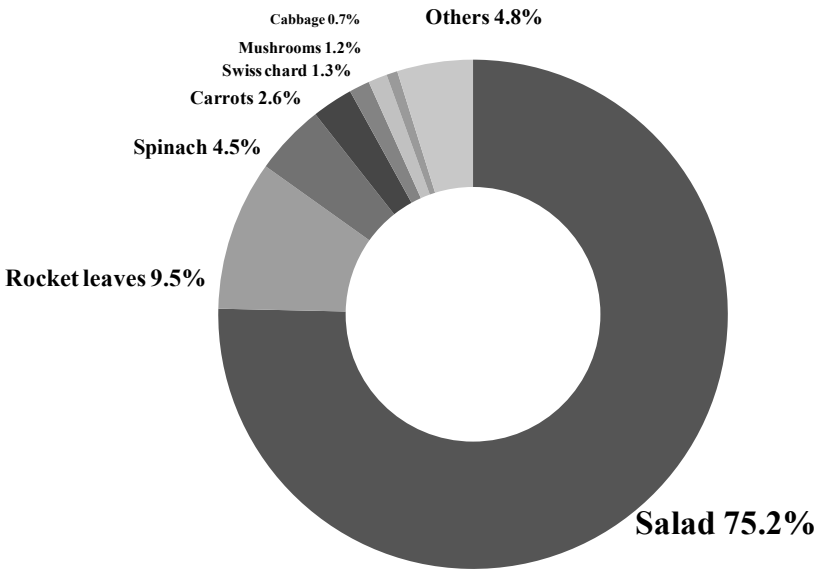

Figure 1.1.1 Fresh-cut market in Italy: products and trends (Adapted from Monitor F&V 2015).

Monitor F&V (2015) also underline that from 2014 to 2015 in Italy there was an increase in consumption of carrots (+10.5% in volume), mushrooms (+8.4), cabbage (+7.1%), and fresh-cut, ready-to-cook, mixed vegetables (+17.8%).

A wide range of commodities are available nowadays for fresh-cut, high-convenience market; among vegetables, fresh-cut salads (iceberg lettuce, romaine lettuce, radicchio), baby leaves (rocket, spinach, valerianella), carrots, potatoes,

onions, tomatoes, zucchini, cabbage, asparagus, celery and zucchini blossoms. Common ready to eat fruits are mainly fresh-cut apples, melon and pineapple (Cantwell and Suslow, 2000; Colelli et al., 2010). Other fruit and vegetables are not yet available for fresh-cut market wither because technological problems related to their quality and safety may have not been solved yet, or because their availability, especially in term of seasonality, does not justify investments in equipment lines and promotional actions. Data reported by Monitor F&V (2015) point out that there is an increase in consumption of new types of fresh-cut products, including artichokes, pumpkins, sprouts and broccoli.

All these data suggest that fresh-cut fruits and vegetables represent a rapidly growing sector in the food industry. The main factor that has promoted and maintain fresh-cut sales is the technology, however permanent innovation are necessary to drive new growth in this sector (Rojas-Graü et al., 2010). Worldwide, there is a wide range of vegetables that could be used to broaden and increase the product offer in the market (Rojas-Graü et al., 2010). Differentiation is a very common approach for presenting higher value added to customers.

One of the most popular vegetable (at least within the Italian market) not presently available as a fresh-cut, high-convenience, ready-to-eat-product, is fennel. As a fresh-cut product fennel would be extremely susceptibility to browning of the cut surfaces, and this might limits its shelf-life. Therefore, in order to remove technological constraints that prevent its availability as a fresh-cut product, an integrated approach would be needed which would take into consideration the

whole production chain, from raw material to processing and packaging in order to address all critical aspects for this kind of production.

1.2. FENNEL AS A FRESH-CUT, HIGH-CONVENIENCE PRODUCT

1.2.1 Botanical characteristic of fennel

Fennel (*Foeniculum vulgare* Mill.) belongs to the family Apiaceae (formerly the Umbelliferae) and it is native to southern Europe and the Mediterranean region (Azeez, 2008). According to USDA-ARS (2013), two subspecies (*Foeniculum vulgare* ssp. *piperitum* (Ucria) Cout. – bitter fennel, and *Foeniculum vulgare* ssp. *vulgare*) are recognised, and the latter subspecies has a number of varieties. Purwaningsih et al. (1999) describe the three varieties of *Foeniculum vulgare* Mill. ssp. *vulgare*: var. *vulgare* (Mill.) (bitter fennel or common fennel) which includes cultivars that have fruits with a bitter aftertaste; var. *dulce* (Mill.) Battand & Trabut, (sweet fennel or Roman fennel) which includes cultivars with sweet-tasting fruits; var. *azoricum* (Mill.) (finocchio o Florence fennel) which includes cultivars with swollen basal part of the petiole which is eaten raw or cooked as a vegetable. Figure 2.1.1 describes the botanical classification of its large and economically important varieties of fennel.

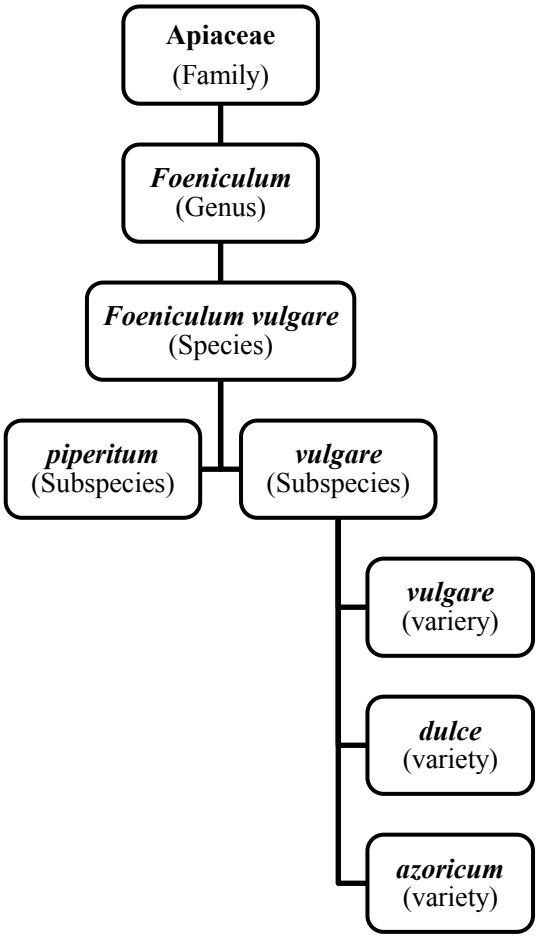

Figure 2.1.1 Botanical taxonomy of fennel varieties (adapted from UDSA-ARS, 2013; Purwaningsih et al., 1999; Azeez, 2008).

Fennel is cultivated as a vegetable crop and also grows wild in Mediterranean countries up to an altitude of over 1000 m and the botanical varieties can be annual, biennial or perennial (Marotti et al., 1993). It is a highly aromatic plant, erect, glaucous green and grows to 2 m tall. The leaves grow up to 40 cm long and they are alternate, decompounds, sheathed, finely dissected (Figure 2.1.2(a)); the lower

leaves are largest and the leaf sheath forming an open cylinder, at base embracing the stem, 2-15 cm long (Figure 2.1.2(b)); the leaf sheath are much larger and fleshier in Florence fennel (Figure 2.1.2(e)). The rest of petiole is subterete, 0-10 cm longer than the sheathing part, longitudinally striate; the blade, triangular in outline, is 2-6-pinnately divided into filiform, blue-green lobes (1-14 cm long and about 0.5 mm wide) and the primary pinnae are odd-numbered 3-19. The flowers are produced in terminal compound umbels 5–15 cm wide, each umbel section with 20–50 tiny yellow flowers on short pedicels (Figure 2.1.2(d)). The fruit is a dry ovoid-cylindrical seed, usually slightly curved schizocarp, from 4–9 mm long, half as wide or less, and grooved. The seeds are light green to yellow-brown when fresh and turn slowly to a dull grey as the seed ages (Figure 2.1.2(c)) (Azeez, 2008; Purwaningsih et al., 1999). The flavour of leaves and seeds is similar to that of anise and star anise, though usually not so strong while the taste of fennel varies from sweet to slightly bitter (Azeez, 2008). *Foeniculum vulgare* Mill. ssp. *Vulgare*, var. *vulgare* and var. *dulce*, are commercially grown for their fruits (seeds) as well as vegetative parts which are used as flavouring, essential oil production or for their medicinal properties (CABI, 2016). The Florence fennel (*Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum*) is smaller than the wild type and is commercially grown for its swollen leaf bases, which form a sort of bulb, and edible leaves which can be eaten raw or cooked as a vegetable. It has a mild anise-like flavour, but is sweeter and more aromatic (Azeez, 2008; CABI, 2016).

Figure 2.1.2. *Foeniculum vulgare* Mill. in its natural habitat (a); stem (b); fennel seeds (c); inflorescences and flowers (d); Florence fennel (e).

1.2.2 Production of fennel

Florence fennel (from now on addressed as "fennel") is a typical crop of Mediterranean area and is grown for the swollen basal part of the leaves (the sheathes), that is thick and crunchy, also called "*grumolo*", but more often referred to as "head". While "grumolo" is the correct botanical term to refer to the fennel unit, the conventional locution "head" will be used hereafter.

Fennel is cultivated in Europe but mostly in Italy, Spain and France (Romano, 2010). In 2008, with almost 500,000 tons cultivated annually on 21,000 ha, Italy largely dominates global production (92% of the total European production), followed by Spain (15,000 MT), France (8,000 MT), Netherland (5,000 MT) and Germany (2,000 MT) (Freshplaza, 2008). The Italian production of fennels from 2010 to 2015 is shown in Table 2.2.1.

Table 2.2.1. Cultivated area (ha) and production of fennel (MT) in Italy in the last 5 years (Data from http://istat.it, 2015).

Year	Total Area (ha)	Total production (MT)
2010	21,588	476.01
2011	21,673	509.78
2012	19,729	489.80
2013	20,760	544.28
2014	19,792	502.78
2015	18,849	502.42

In Italy fennel is mainly cultivated in Puglia (5,890 ha and 128,810 MT), representing 25.6% of the total cultivated area, followed by Calabria and Campania (Table 2.2.2).

Table 2.2.2. Cultivated area (ha) and production (MT) of fennels in Italian regions (Data from http://istat.it, 2015).

Region	Total Area (ha)	Total Production (MT)
Puglia	5,890	128,810
Calabria	2,204	84,776
Campania	2,876	81,344
Abruzzo	2,447	61,804
Molise	1,000	34,000
Sicilia	1,528	32,615
Sardegna	827	26,866
Basilicata	796	20,280
Lazio	610	13,300
Emilia-Romagna	197	6,552
Toscana	208	4,307
Marche	122	4,079
Piemonte	69	1,645
Veneto	49	1,432
Friuli-Venezia Giulia	10	258
Liguria	7	190
Lombardia	6	108
Umbria	3	53
Valle d'Aosta	-	-
Trentino-Alto Adige	-	-
Bolzano	-	-
Trento	-	-
ITALY	**18,849**	**502,417**

In Puglia fennel is mainly cultivated in the province of Foggia and Bari (Table 2.2.3).

Table 2.2.3. Cultivated area (ha) and production (MT) of fennels in the provinces of the Apulia Region (Data from http://istat.it, 2015).

Province	Total Area (ha)	Total Production (MT)
Foggia	2,500	47,500
Bari	1,400	28,000
Taranto	500	15,000
Brindisi	800	22,000
Lecce	310	8,370
Barletta-Andria-Trani	380	7,940
Total Puglia	**5,890**	**128,810**

The cultivation of fennels generally starts with the transplantation of the seedlings in open field and the time of transplantation depends on climatic conditions. Even if fennel is a typical winter crop, different cultivars have been selected, allowing the cultivation and the harvest of fennels almost in all time of the year. There are three main categories of cultivars, based on the seedlings transplantation period and on the time that occurs for the complete growth of the crop.

Early cycle cultivars: are cultivars for summer production. The seedlings are transplanted from April to June and fennels are harvested after 85 – 95 days. Fennel heads are generally medium size (400-450g).

Medium cycle cultivars: are cultivars for autumn – winter production. The seedlings are transplanted in July – August in the North Italy and in central regions, in late August – early September in the South Italy and on the coasts. Fennel heads are harvested after 120 – 160 days.

Late cycle cultivars: are cultivars for winter – spring production. The seedlings are transplanted from September to October in the South Italy and on the coasts. Fennel heads are harvested after 130 – 200 days, reaching generally large size (500-700g).

Using different cultivars, farmers are able to provide fresh fennels almost in all period of the year. Despite the growing time being specific for each fennel cultivar, climatic conditions during the growth of the plant may influence the time of harvest, therefore the harvest may be anticipated or postponed in order to satisfy the quality parameters required by the market. No specific horticultural maturity index are available on fennel, however according to Mencarelli (2004) and Romano (2010) fennel is harvested by hand when the plant reaches the full size (the size depend on cultivar), the sheathes should be tightened, swollen and white.

The United Nation Economic Commission for Europe (UNECE) provide a list of standard concerning the marketing and commercial quality control of fennel or varieties (cultivars) grown from *Foeniculum vulgare* var. *azoricum* (Mill.) Thell (UNECE STANDARD FFV-16, 2013). This standards can be applied only for fresh consume, and fennel heads for industrial processing are excluded. Heads must be intact, however roots and leaves must be trimmed. According to UNECE STANDARD (UNECE STANDARD FFV-16, 2013), fennels are classified in two classes, as shown in Table 2.2.4.

Table 2.2.4. Classification of fennel according to UNECE STANDARD (UNECE STANDARD FFV-16, 2013).

	Quality standards requested	Defects allowed
Class I	All the quality standards requested for Class II. Regular shape, compact outer ribs, fleshy, tender, white.	Slight defect in shape, slight bruising, slight healed and not discolored cracks.
Class II	Clean, free of any visible foreign matter, free from pests and damage caused by pests, fresh appearance, firm, not running to seed, free from damage caused by frost, and free of abnormal external moisture and of any foreign smell and/or taste. Roots must be severed close to the base of the bulbous part and the length of the leafy ribs of the heart must be not exceed 7 cm.	Defects in shape, bruising, healed cracks not exceeding 3 cm in length, green patches on the outside of the bulb, covering not more than one-third of its surface (only to the outer ribs).

The quality standards for fennels of Class II are the minimal standards required by UNECE STANDARD FFV-16 (2013). Regardless of the class, the development and condition of the fennel must be such as to enable them to withstand transportation and handling and to arrive in satisfactory condition at the place of destination (UNECE STANDARD FFV-16, 2013).

Quality of fennel depends on a combination of characteristic, such as appearance, flavor, texture, and nutritive value. High fennel quality is first related to its uniform and brilliant white appearance. The fennel culinary value is related to its organoleptic properties such as taste, aroma and aniseed-flavour but also to its crunchiness (Barros et al., 2010; Mencarelli, 2004). The typical fennel taste is due to the balance of its sweetness, thanks to good sources of carbohydrates, such as fructose, glucose, sucrose and mannose (Cataldi et al., 1998; Escalona et al., 2006;

Barros et al., 2010), and the acidity, mainly due to the presence of oxalic and malic acids (Escalona et al., 2006; Sánchez-Mata et al., 2012; Pereira et al., 2013). The characteristic aroma in fennel is related to its richness in essential oils and volatile compounds, mainly *trans*-anethole and fenchone but also estragole, p-anisaldehyde and terpenes, that give the characteristic 'anise' flavour (Badgujar et al., 2014; Yadav and Malik, 2015). Another important sensorial attribute that is really appreciated by consumers is the crunchiness. The leafy sheathes of fennel are turgid, and have a crispy texture because of the high turgor pressure of the plant tissues that have high water content and are rich in fibers and soluble solids. Since the amount of water, fiber and soluble solid, changes among the layers of sheathes that surrounded the stem, these latter have different texture characteristics. From the nutritional point of view fennel is characterized by a very low energy content (9 kcal 100 g^{-1} fw) therefore it can be considered an hypocaloric food that has a high fiber content (2.2 g 100 g^{-1} fw). In addition fennel is a good source of potassium (394 mg 100 g^{-1} fw) and vitamin C (12 mg 100 g^{-1} fw). A daily consumption of 100 g of fennel provide 20% of the recommended dietary allowance (RDA) for vitamin C. The nutritional composition of fennel is shown in Table 2.2.5.

Table 2.2.5. Nutritional composition of fennel. Data from INRAN.

Chemical composition	Values refers to 100 g of edible part
H_2O (g)	93.2
Proteins (g)	1.2
Total carbohydrates (g)	1.0
Starch (g)	-
Soluble sugars (g)	1.0
Total fibres (g)	2.2
Soluble fibers (g)	0.25
Insoluble fibers (g)	1.97
Potassium (mg)	394
Calcium (mg)	45
Phosphorus (mg)	39
Vitamin C (mg)	12
Energy (kcal)	9

Generally, the most used instrumental techniques to measure quality attributes of fruits and vegetables are destructive and involve a considerable amount of manual work, primarily due to sample preparation. In addition, most of these analytical techniques are time consuming, and sometimes, may require sophisticated equipments (i.e. the analyses of phenolic compounds and antioxidant activity by spectrophotometer, sugars, organic acids, and vitamin C by HPLC). Moreover, destructive analyses can be performed only on a limited number of specimens and, thus, their statistical relevance may be limited. In recent years, researches have been focused on the development of non-destructive techniques suitable to increase the number of specimens that can be analysed, to repeat more times the same analysis

on the same sample at a given time or during its physiological evolution, and in order to achieve real-time information (Costa et al., 2009).

Hyperspectral imaging, among non destructive spectral techniques, has gained importance in the past few decades due to its potentiality for accurate, robust and non-destructive prediction of food quality of different types, including fruits and vegetables. Previous studies have successfully used hyperspectral imaging for the evaluation of composition and quality of many crops as apples (Bobelyn et al., 2010), strawberry (Choudhary et al., 2010), oranges (Cayuela, 2008), mangoes (Jha et al., 2012), melons (Flores et al., 2008), pineapples (Chia et al., 2012), tomatoes (Peiris et al., 1998) and many other. A few scientific studies have been carried out to determine the anethole content in fennels (Baranska et al., 2004), determination of fennel chemotypes (Gudi et al., 2014), essential oil content of fennel (Schulz et al., 2000, Strehle et al., 2005) by using different spectroscopic techniques such as NIR-FT-Raman microspectroscopic mapping, IR-Raman spectroscopy and FT-NIR, however all of these studies were carried out on fennel fruits.

On the other side there is a lack of literature in studies aimed to predict fennel head composition in terms of maturity-related attributes, also to better assess the harvest time and the internal organoleptic and nutritional properties. In general few studies are addressed to discriminate among fruits and vegetable from different harvest times, and mainly for wine (González-Caballero et al., 2012) and table (Piazzolla et al., 2013) grapes. Given these considerations, the use of hyperspectral imaging could be a suitable approach to predict quality attributes in fennel heads.

1.2.3 Postharvest handling of fennel

Once the crop is harvested it still continues its biological processes until it deteriorates to an unsalable product. The key to successful postharvest handling is to delay these processes in order to get the product to the consumer in the best possible condition. Very few information about postharvest handling of fennel are available in literature. It is reported that fennel is highly sensitive to physical injury, therefore special attention must be given during postharvest handling; removal of the outer sheathes at retail markets reduces the problem, but the process is time consuming (Mencarelli, 2004). After harvest fennels are sized by the packer and placed in plastic or cardboard boxes (Mencarelli, 2004). According to Sozzi et al. (1992) hydro-cooling is needed in summer to reduce water loss and field heat. The same authors also recommend to use forced-air cooling if fennels are packed in plastic wraps. Fennel has moderate respiration rate (see Table 2.3.2) and shows a typical non-climacteric pattern (Artés et al., 2002a).

Table 2.3.2. Respiration rate and heat production in fennels at different storage temperature (data from Mencarelli, 2002 and Artés et al., 2002).

Temperature (°C)	**Respiration rate (mg CO_2 kg^{-1} h^{-1})**	**Heat production (cal kg^{-1} h^{-1})**
0	9 - 12	23 - 30
2 – 5	18 - 20	46 - 51
20	24 - 40	61 - 102

Escalona et al. (2004) showed that respiration rate of fennels decrease from 14 to 8 mg CO_2 kg^{-1} h^{-1} after 8 days of storage at 0 °C; the same pattern is followed by ethylene production.

Temperature is the most important factor affecting postharvest life of horticultural crops. This is because temperature has a profound effect on the rates of biological reactions, as metabolism and respiration. Exposure of the commodities to undesirable temperatures can result in physiological disorders (Table 2.3.3).

Table 2.3.3. Effect of temperature on physiological breakdown (Kader, 2002a).

Temperature	Physiological breakdown	Alterations
< Freezing point	Freezing injury	Collapse of the tissue and total loss of the commodity
> Freezing point 5-15 °C	Chilling injury	Browning, pitting, watersoaked areas, uneven ripening, off-flavour, accelerated incidence of surface mould and decay
Very high temperature	Heat injury	Bleaching, surface scalding, uneven ripening, desiccation

Fennel is not chilling sensitive; the highest freezing temperature is -1.1 °C. A storage temperature of 0 to 2 °C (32 to 36 °F) is recommended in order to extend postharvest life (Cantwell, 2001; Mencarelli, 2004). Another important factor that affects postharvest life of horticultural crops is the relative humidity (RH). Relative humidity can influence water loss, decay development, and incidence of some physiological disorders. Condensation of moisture on the commodity over long periods of time is probably more important in enhancing decay than is the RH of ambient air (Kader, 2002a). Management of RH is very important for fennels; Mencarelli (2004) suggests to keep fennels refrigerated and periodically moistened

with water spray. Ideally, storage rooms should operate at 0 to 2 °C with 90-95% RH; in these conditions fennels can be stored for 2 – 3 weeks (Cantwell, 2001).

The postharvest life of horticultural crops can be further extended through modification of the atmosphere surrounding the product, (Zagory et al., 1988; Kader, 2002a). Modified (MA) and controlled atmospheres (CA) usually involve a reduction of O_2 and/or an increase in CO_2 levels (Yahia, 2009) and are a very useful supplement to providing the proper temperature and relative humidity, reduce respiration rate and weight loss, to delay ripening and softening, and could minimize the incidence of some physiological disorders and decay (Kader, 2002b).

For fennels, the application of atmosphere modification is recommended to delay browning of the butt-end cut zone of fennel heads. At harvest fennels are trimmed, cutting the roots on the basal portion of the swollen head; after this operation the butt end cut zone rapidly turn brown representing the most important factor affecting visual quality and reducing the shelf-life of fennel (Artés et al., 2002a).

Controlled atmospheres of 5 kPa O_2 and 5 kPa O_2 + 5 ka CO_2 were able to maintain the visual quality for 11 days at 0 and 5 °C, with an additional storage at 15 °C for 3 days, reducing the browning of the butt end cut zone as well as on the external leaves of “Orion” fennels compared to control in air. Similar results were obtained on fennel cv. “Clio” stored in CA of 5 kPa O_2 + 5 ka CO_2 and 5 kPa O_2 + 20 ka CO_2 at 0 °C for 14 days followed by complementary 3 - 4 days at 15 °C (Artés et al., 2002a). A reduction of respiration rate and a delay of browning of the butt-end cut zone of fennel heads cv. “Orion” were observed after 28 days at 5 °C in CA of 5 kPa O_2 + 5 ka CO_2 and after 21 days at the same temperature if the CA applied was

5 kPa O_2 + 15 ka CO_2 (Escalona et al., 2006). A passive MAP, using basket or bags of unperforated polypropylene, allowed to preserve the visual appearance of fennel bulbs stored for 14 days at 0°C followed with complementary 3 days in air at 15°C (Escalona et al. 2004). The application of antioxidant solutions (1% ascorbic acid or 5% citric acid) on the butt-end cut zone of fennel heads resulted to have no effect on delaying browning after 14 days at 0°C in PMAP, rather they caused bulb softening (Artés et al., 2002b).

No study regarding the application of active MAP on fennel are reported.

1.2.4 Fresh-cut fennel

The development of new products for fresh-cut market requires the knowledge of postharvest handling of the intact crop under study, however fresh-cut products differ from traditional intact ones in term of their physiology and their handling requirements. Fresh-cut produce is essentially purposely wounded plant tissue that must subsequently be maintained in a viable, fresh state for extended periods of time. Fresh-cut vegetables deteriorate faster than intact produce. This is a direct result of the wounding associated with processing, which leads to a number of physical and physiological changes affecting the viability and quality of the produce (Brecht 1995; Saltveit 1997a). To minimize the loss of quality of fresh-cut products in terms of appearance, texture, flavor, and nutritive value the selection of raw materials is of paramount importance. Only fruit and vegetables of the best quality, in terms of development, physiological condition, appearance and integrity, can hold up the stress induced by processing, maintaining high quality until the end of

their commercial life (Colelli and Elia, 2009). Among quality attributes that are influenced by processing for fresh-cut fennel, the appearance is that most relevant because it is the attribute most immediately obvious to the consumer, strongly affecting the decision to buy (Toivonen and Brummel, 2008). As for whole fennels on the butt-end cut zone, for fresh-cut fennel the most important factor that influences quality, limiting its shelf life, is the browning of the cut surfaces (Albenzio et al., 1998; Artés et al., 2002a,b; Escalona et la., 2005a,b); therefore for the developing of fennel as fresh-cut product all the parameters that may contribute to onset of this problem should be considered.

An important factor that determines storage life and the final qualities of a product, since affect the intensity of the wound response in fresh-cut vegetables and fruits, is the maturity stage at harvest (Kader, 2002a; Toivonen and DeEll, 2002; Brecht et al., 2004; Beaulieu, 2010). In general, for fresh-cut processing, the optimum harvest time depends on the type of commodity. In climacteric fruits the optimal harvest time is usually slight before the full maturity stage in order to avoid loss of firmness that could reduce the storability of the products (Colelli and Elia, 2009). Non-climacteric commodities, as in the case of fennel, do not ripen further after harvest, so harvesting at the proper stage of maturity is essential for optimal quality in terms of appearance, nutritional, and sensorial attributes. The investigation of the optimal harvest time is especially important for fennels since no maturity standards are available for this crop, except for product size and appearance.

The selection of the appropriate maturity at harvest is a pre-harvest factor that should be taken into account in order to minimize the loss of quality of fresh-cut

fennels. However the occurrence of browning could be controlled after cutting operations using different approaches. One of them is the use of surface treatments that consists in dipping the slices of the fresh-cut product in aqueous solutions containing antibrowning agents. Browning of the cut surface of fresh-cut products is mainly caused by oxidation of phenolics to o-quinones, catalyzed by the oxidative enzymes, including polyphenol oxidase (PPO) and peroxidases (POD). Quinones then polymerize to form dark pigments, leading to browning appearance. The antibrowning agents used to control surface discoloration generally act directly on the enzyme (i.e. PPO), as enzyme inhibitors, others by rending the medium inadequate for the development of the browning reaction, while others by reacting with the products of the enzymes reaction before the formation of dark pigments (Garcia and Barrett, 2002). Ascorbic acid is on of the most extensively used agent to avoid enzymatic browning and act by reducing the quinone products to their original polyphenol compounds and, to a lesser extent, as an acidulant (Walker, 1977; Garcia and Barrett, 2002). Citric acid is a strong acidulant and can inhibit the PPO by lowering the pH below that necessary for the optimal enzyme activity; in addition citric acid can inhibit PPO working through a non-competitive mechanism, by chelating copper at the enzyme active site (Ibrahim et al., 2004; Altunkaya et al., 2008; Ali et al., 2015). L-cysteine can inhibit the browning by trapping *o*-quinones through the formation of cysteinyl adducts or reducing *o*-quinones to their polyphenols precursors (Richard-Forget et al., 1992; Cilliers and Singleton 1990). However the effectiveness of L-cysteine as antibrowning is largely influenced by pH. In a study on fresh-cut artichoke Cabezas-Serrano et al. (2013) used L-cysteine

solutions at the same concentration but at different pH (from 2.2 to 7) and results revealed that L-cysteine at pH 7 was most effective in control browning than low pH cysteine solutions. 4-hexylresorcinol is another compound used as antibrowning agent since it is a competitive inhibitors of PPO: it interacts with PPO to render an inactive complex incapable of catalyzing the browning reaction (Whitaket et al., 1995; Lambrecht, 1995). A wide range of studies have evaluated the efficacy of different antibrowning agents, alone or in combinations, on fresh-cut fruits and vegetables, (Monsalve-González et al., 1993; Luo et al., 1995; Sapers et al., 1998; Moline et al., 1999; Dong et al., 2000; Chiesa et al., 2001; Gorny et al., 2002; Ibrahim et al., 2004; Amodio et al., 2011; Pace et al., 2014; Wang et al., 2014). Regarding the application of antibrowning reagents to reduce the browning on fennels, very few investigation are available. Artés et al. (2002b) reported treatments with 1% ascorbic acid and 5% citric acid that however did not control browning of butt-end cut surface of whole fennels. Rinaldi et al. (2007) applied pre-treatments with citric acid, ascorbic acid, sodium chloride, cysteine and ethanol solution, alone on in various combination, on fresh-cut fennels and, among them, dipping for 1 minute in 0.5% ethanol solution slightly delayed browning, while dipping for 1 minute in 1% citric acid + 0.5% cysteine solution, adjusted at pH 7, did not statistically differ from control. In addition dipping in all the other solutions produced a more severe browning than control. On the other hand Albenzio et al. (1998) investigated the effectiveness of citric acid solution at different concentrations applied for 15 or 30 min, concluding that dipping fresh-cut fennels for 15 min in 0.1% citric acid is useful to delay the occurrence of browning of 5 day

at 4 °C. Starting from these results, further investigations regarding the use of antibrowning agent on fresh-cut fennel should be done.

Another common approach used to increase the shelf-life of fresh-cut products is the modification of the atmosphere that surrounds the commodities. The suitable gas mixture for modified atmosphere has been based on that recommended for the whole commodity (Saltveit, 1997b). However fresh-cut products probably can tolerate more extreme levels of O_2 and CO_2, because they do not have as much cuticle or skin to restrict gas diffusion, and the distance of gas diffusion from the center to the outside of the product is much lower than that for the whole commodity (Watada and Qi, 1999). Therefore specific investigations are needed to select optimal O_2 and CO_2 concentrations for a given fresh-cut product. Usually controlled atmosphere system is used to simulate modified atmosphere with similar gas composition for assessing quality (Watada and Qi, 1999). Escalona et al. (2006) reported that a CA condition with 5 kPa O_2 in combination with 5 or 15 kPa CO_2 is recommended for keeping quality of sliced fennels for 14 days at 5 °C even though gas composition of 5 kPa O_2 + 15 kPa CO_2 delayed the browning of the cut zone for longer time compared to 5 kPa O_2 + 5 kPa CO_2. Rinaldi et al. (2010) reported that a CA condition with 10 kPa CO_2, in air or with 5 kPa O_2, was able to extent the shelf-life of fennel quarters until 12 days at 5 °C compared to a CA with 5 kPa O_2 or air. No further studies are available regarding CA of fresh-cut fennels therefore it will be useful to investigates the effect of wider ranges of gas compositions, such as lower oxygen and higher CO_2 levels, on color changes in fresh-cut fennel during storage. Using CA is possible to maintain the optimal gas compositions selected for

each product during time. However if the final goal for a fresh-cut product is the commercialization for consumers, the optimal packaging conditions should be investigated. Modified atmosphere packaging (MAP) is an effective tool to maintain quality of fresh-cut products through its effects on modification of the gas composition in the package headspace (Schlimme and Rooney, 1994; Jacxsens et al., 2002; Kim et al., 2003, Luo et al., 2004). Benefits of film packaging, other than creation of MA conditions can include maintenance of high relative humidity with consequent reduction of water loss (Kader and Watkins, 2000). The basic premise of MAP technology is that once produce is placed in a package and hermetically sealed, an environment different from ambient conditions will be established (Gorny, 1997). For fresh-cut fennels limiting studies regarding the use of MAP are available. Albenzio et al. (1998) reported that the shelf-life of fresh-cut fennels can be prolonged up to 10 days at 4 °C using a vacuum-packaging combined with a pretreatment with 0.1% citric acid for 15 min. Diced fennels cv. Orion stored at 0 °C for 14 days in polypropylene basket sealed with non-perforated polypropylene film or in polypropylene bags, generated a passive atmosphere of 11-13 kPa O_2 and 9-12 kPa CO_2 that was not useful to delay the browning of the cut surface (Escalona et al., 2005a). Better results were obtained on fennel slices cv. Clio stored in polypropylene (PP) trays and sealed with non-perforated polypropylene film (OPP) where a passive atmosphere of 4-6 kPa O_2 and 10-14 kPa CO_2 was generated, allowing to prolong the shelf-life at 0 °C for 14 days (Escalona et al. 2005b). In the same experiment none of the conditions tested at 5 °C (PP trays sealed with OPP film unperforated, and with one or two perforations) was able to inhibit the

browning of the cut surface of fresh-cut fennel. Starting from these results, further investigation regarding the use of MAP technology are necessary to better optimized packaging conditions for fresh-cut fennels, especially at 5 °C as usual commercial temperature.

In general fresh-cut products are very perishable. Previous investigations on fresh-cut fennels revealed that its shelf-life is very limited, mainly due to the browning that occurs on the cut-surface. The application of pretreatments and the modification of the atmosphere during cold storage can delay the occurrence of this disorder, however the optimization of the critical aspects that may influence the loss of quality in fresh-cut fennel could allow to increase the shelf-life of this product.

The prediction of the shelf-life for fresh-cut fennel as in general for fresh-cut products has become crucial for both processors and consumers to improve the produce logistics all over the chain and ensure lower costs and a better final quality of the product. Different approaches has been developed in order to predict the shelf-life. One of the common used method is the Accelerated Shelf Life Testing (ASLT) proposed by Labuza (1982), that although enables the calculation of a suitable shelf life estimation, cannot assure agreement between what is estimated and what is observed experimentally. In addition when the quality of food is defined by multiple attributes, the use of the common ASLT may produce significant discrepancies. Another approach recently used for determining shelf life is the Multivariate Accelerated Shelf Life Testing (MASLT) proposed by Pedro and Ferreira (2006) that, taking into account many quality attributes simultaneously, allow to obtain a more realistic shelf-life estimation of a fresh-cut product (Derossi

et al., 2016). Thus, beside the optimization of the main aspects that may induce a loss of quality in fresh-cut fennel, innovative approaches that allow to estimate the degradation of the quality parameters during different storage conditions, can be applied to better define the shelf-life of the products, with possible advantages for processors and consumers.

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PART TWO: EXPERIMENTAL

2.1 GENERAL OBJECTIVES

Fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) is one of the most popular vegetable in Italy that is commonly eaten raw either alone or mixed in a salad, or cooked as vegetable. Consumers mainly appreciates this crop for its organoleptic properties such as sweet-taste, aroma and aniseed-flavour but also for its crunchiness. However the high percentage of discarded plant waste, together with complex and time-consuming trimming operation, may discourage consumers, affecting their decision to buy. Consumers often have limited time to spent purchasing, storing, preparing and consuming food. In addition they are more concerned about the nutritional, sensorial and safety aspects of the food they eat due to growing health awareness.

From these consideration, fresh-cut processing is very desirable for fennel, since it is still not available as a fresh-cut, high-convenience, ready-to-eat-product. As a fresh-cut product fennel would be extremely susceptible to browning of the cut surfaces, and this might limits its shelf-life. Therefore, in order to remove technological constraints that prevent its availability as a fresh-cut product, an integrated approach is needed taking into consideration the whole production chain, from raw material to processing and packaging in order to address all critical aspects for this kind of production.

Through the execution of a number of experimental trials aimed to optimize most important processing steps for fennel as a fresh-cut product, the following objectives will be pursued:

- evaluate the effect of maturity at harvest on quality characteristic and chemical composition of fennels and their browning susceptibility when processed as a fresh-cut products;
- detect the capability of Vis/NIR spectroscopy for the prediction of quality attributes of fennels at different harvest times;
- investigate the effectiveness of different antibrowning solutions on maintaining quality characteristics of fresh-cut fennel during storage;
- identify best suitable gas mixture to extend the shelf-life of fresh-cut fennel;
- design a modified atmosphere packaging to extend the shelf-life of fresh-cut fennel.

2.2 METHODS

2.2.1 Quality attributes

2.2.1.1 Respiration rate and weight loss

Respiration rate was measured using a dynamic system (Kader, 2002b). At each sampling time 0.5 mL gas samples were collected from the inlet and from the outlet flows of each jar and injected into a gas chromatograph (Shimadzu, model 17A, Kyoto, Japan) equipped with a thermal conductivity detector (200 °C). Separation of CO2 was achieved on a Carbonex 1006 plot (30 m x 0.53 mm, Supelco, Bellafonte, PA, USA), with a column flow of 7 mL min-1, and over temperature of 180 °C. Calculations of respiration rate (in mL CO_2 $kg^{-1}h^{-1}$) were based on the differences in CO_2 concentration, referred to the sample weight and to the air flow rate.

Fennel weight loss was calculated at sampling dates as percentage of variation from the initial fresh weight.

2.2.1.2 O_2 and CO_2 headspace analysis

In MAP experiments, O_2 and CO_2 levels in the package headspace were measured during storage using a handheld gas analyser (CheckPoint, Dansensor A/S, Denmark). The apparatus is based on an electrochemical sensor to record the O_2 content and on a mini-IR spectrophotometer to record the CO_2 content in the

package (accuracy: 0.1% O_2; 2% CO_2). The instrument was calibrated with O_2 and CO_2 air percentages.

2.2.1.3 Color

Color of fennel slices was measured elaborating the images acquired with a Spectral Imaging spectrometer (DV SRL, Padova, Italia) V10 type (400-1000nm, 25 μm slit, resolution 5nm). One scan of 8 random slices per replicate was acquired with a speed of 3 mm min^{-1} in a dark room with a stabilized halogen light source (150 W). On each fennel slice, regions of interest (ROI), separately on the stem and on the sheath, were manually selected as the maximum subscribed rectangle, allowing to calculate in the reflectance mode, the CIE L*, a* b* scale color parameters. The L* value represents lightness; the +a* and –a* values represent redness and greenness, respectively. The +b* and –b* values represent yellowness and blueness, respectively. Hue angle ($h° = \arctan \frac{b^*}{a^*}$), saturation ($Chroma = \sqrt{a^{*2} + b^{*2}}$) and total color variation ($\Delta E = \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2}$) were then calculated from primary L*, a* and b* readings.

2.2.1.4 Sensorial analysis

The sensorial attributes of fennel samples were observed at each sampling time by a trained panel of five members. The appearance was scored using a scale from 5 to 1, where 5 = excellent (fresh appearance, bright white color), 4 = good (slight dehydration on the cut surfaces, very mild signs of deterioration of the stem and absence of defects in the sheath, slight yellowing), 3 = fair (noticeable dehydration

on the cut surfaces and yellowing, appreciable signs of deterioration on the stem and mild on the sheath), 2 = poor (severe symptom of dehydration and perceptible deterioration on stem and on the sheath, appreciable browning of the stem), 1 = very poor (severe dehydration on stem and sheath, severe browning of the stem, possible microbial spoilage). A score of 3 was considered as a limit of marketability while a score of 2 was considered as a limit of edibility (Figure 2.2.4.1). Browning of the cut surfaces was scored separately on stem and sheath parts of sliced fennel using the scale from 1 to 5, where 1= absence of browning, 3= slight browning, 5= complete browning. The same 5 point scale structure was used to evaluate subjectively the other attributes such as aroma (1= absent, 3= moderate, 5= full characteristic), crunchiness (1= not crunchy, 3= fairly crunchy, 5= very crunchy), dehydration (1= fresh-like, 3= slightly dehydrated, 5= very dehydrated), flavor (1= absent, 3= moderate, 5= full characteristic) and sweetness (1= not sweet, 3= slightly sweet, 5= very sweet). Finally, on the base of all these sensorial parameters, panelists attributed an overall evaluation using a scale from 1 to 5, where 1= very poor, 3= fair, and 5= excellent.

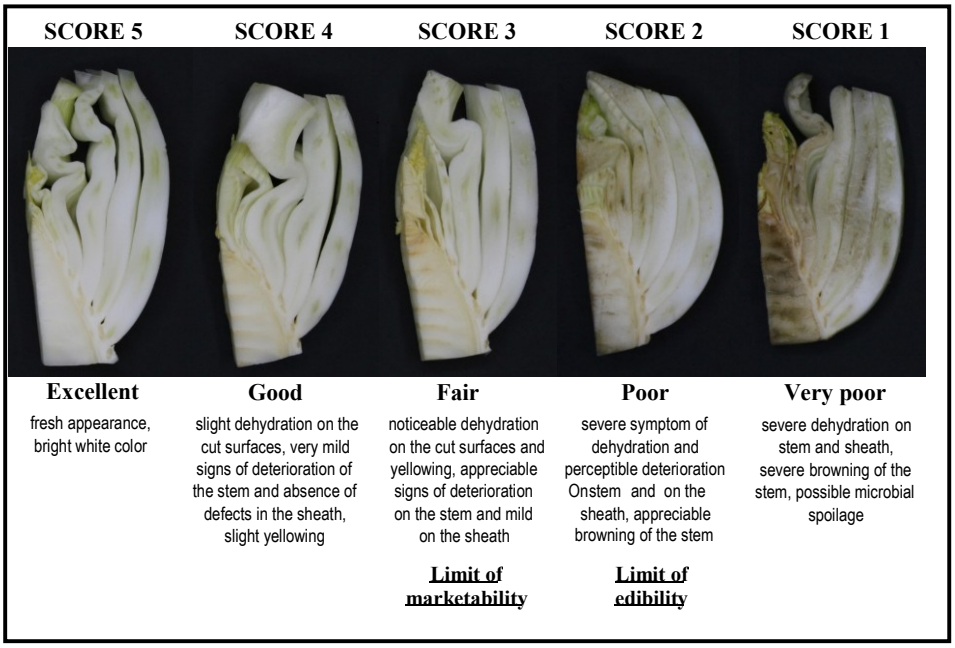

Figure 2.2.1.4.1. Rating scale for fresh-cut fennels

2.2.1.5 Total soluble solids, titratable acidity, and pH

For the measurement of total soluble solid (TSS), titratable acidity (TA) and pH, 20 g of fennel tissues were transferred in a falcon tube, homogenized in an Ultra-Turrax (IKA T18 basic, Wilmington, NC, USA) and filtered with two layers of cheesecloth. Few drops of the fennel juice obtained were used to measure TSS content with a digital refractometer (Atago PR32-Palette, Tokyo, Japan). Another fraction of 1 g of fennel juice was used to measure the pH and the TA (reported as mEq NaOH 100 g^{-1} of fresh weight), with an automatic titrator (Titrator T50, Mettler Toledo) titrating with NaOH 0.1N until final pH of 8.1.

2.2.1.6 Total phenolic content and antioxidant activity

The same extraction was carried out for analyses of total phenolic content and antioxidant activity, following the procedure described by Amodio et al. (2014) with slight modifications. Five grams of fresh fennel tissue were homogenized in 2 mM sodium fluoride (NaF) methanol:water solution (80:20) for 1 min, using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA). The homogenate was filtered through two layers of cheesecloth and then centrifuged (PK 121R, Thermo Electron Corporation, France) at 10,000 rpm for 10 min at 4 °C. The pellet was discarded and the supernatant was retained and used as the extract. The total phenolic content was determined according to the method reported by Singleton and Rossi (1965). Each extract (100 μL), appropriately diluted, was mixed with 1.58 mL distilled water, 100 μL of Folin-Ciocalteau reagent and 300 μL of a sodium carbonate solution (Na_2CO_3 200 g L^{-1}). After 2 h of incubation at room temperature in the dark, the absorbance was read at 725 nm against a blank using a spectrophotometer (Shimadzu UV-1700, Jiangsu, China). The total phenolic content was calculated based on the calibration curves of gallic acid (0-500 μg/ml) and expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} fw). Antioxidant activity was performed following the procedure described by Brand-Williams et al. (1995) with minor modifications. Each extract (50 μL), appropriately diluted, was mixed with 950 μL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after overnight incubation at room temperature in the dark. Antioxidant activity was calculated as mg of Trolox equivalents per 100 g of fresh weight (mg TEAC 100 g^{-1} fw) using a Trolox standard curve (0-625 μM).

2.2.1.7 Vitamin C

Vitamin C content was assessed homogenizing 10 grams of fennel tissue in an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 2 min with 10 mL of methanol/water (5:95), plus citric acid (21 g L-1), EDTA (0.5 g L-1) and NaF (0.168 g L-1). The homogenate was filtered through two layers of cheesecloth and the pH was adjusted to 2.2 – 2.4 by addition of 6 N HCl. After centrifugation (PK 121R, Thermo Electron Corporation, France) at 12,000 rpm for 5 min at 4 °C, the supernatant was filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then though a 0.2 μm cellulose acetate filter (INCOFAR, Modena, Italy). L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA) contents were determinate as described by Zapata and Dufour (1992) with some modifications (Gil et al., 1999). The HPLC analysis was achieved after derivatisation 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 analysed with an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB - C18 column (150 mm x 4.6 mm; 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was methanol:water solution (5:95 v/v) containing 5 mmol L^{-1} cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. The detector wavelengths were 348 nm for DHA and 251 nm for AA. AA and DHAA contents were expressed as mg of L-ascorbic or L-dehydroascorbic acid $100g^{-1}$ of fresh weight.

2.2.1.8 Simultaneous analysis of organic acids and sugars

Organic acid and sugars were extracted homogenizing 10 g of fresh fennel tissue with 20 mL of ultrapure water using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) at 14000 rpm for 1 min. The homogenate was centrifuged at 9000 rpm for 10 minutes at 5 °C. The supernatant was filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then though a 0.2 μm cellulose acetate filter (INCOFAR, Modena, Italy). All extract were performed in triplicate samples. Organic acid and sugars were identified using the method described by Mena et al. (2011). 10 μL of samples were injected into HPLC system (Agilent 1200 series) equipped with an UV detector, set at 210 nm, coupled with a refractive index detector. Peak separation was achieved on a Rezex ROA-Organic Acid H+(8%) column (300 x 7.80 mm) (Phenomenex, Torrance, USA), using a mobile phase of acidified water (phosphoric acid 0.1%) with a flow rate of 0.5 mL/min and a oven temperature of 30 °C. The different organic acid and sugars were characterized and quantified by chromatographic comparison with analytical standards. Results of organic acid and sugars content were expressed as mg $100g^{-1}$ of fresh weight and g $100g^{-1}$ of fresh weight respectively.

2.2.1.9 Acetaldehyde and ethanol

Acetaldehyde and ethanol content was assessed according to Mateos et al. (1993). Five g of fresh fennel tissue was homogenized and put into 22 mL glass test tube, sealed with rubber stopper and stored at -20 °C freezer until analysis. After 1 hour incubation at 65 °C water bath, a 0.5 mL headspace gas sample was taken and

injected into a gas chromatograph (Shimadzu GC-14A; FID temperature was 150 °C, injector temperature was 130 °C, oven temperature was 80 °C. 5% CBWX 20M on Carbograph 1AW20 80/120, 6' x 1/8" x 0.085" AT STEEL column (Alltech). Acetaldehyde and ethanol were identified by co-chromatography with standards and quantified by a range of concentrations of acetaldehyde and ethanol in 5 mL of water. Acetaldehyde and ethanol concentrations were reported in μmol g^{-1} of fresh weight.

2.2.1.10 Microbiological analysis

Two slices of fennel for each replicate were weighted, diluted (1:10) in a sterile saline solution (NaCl 9 g L^{-1}), and homogenized for 2 min in a blender (Bag Mixer, Interscience, Saint-Nom-la-Bretèche, France), using sterile filter stomacher bags (BagFilter®, Interscience, Saint-Nom-la-Bretèche, France). Tenfold serial dilutions were made in a sterile saline solution as required for plating. Total aerobic mesophilic and psychrophilic bacteria were enumerated using plate count agar (PCA) (Oxoid, Basingstoke, Hampshire, UK) after incubation at 30 °C for 48 h and at 5 °C for 7 days respectively. Enterobacteriaceae were counted in Violet Red Bile Agar (VRBA) after incubation at 37 °C for 24 h. Lactic acid bacteria were counted in de Man Rogosa Sharpe (MRS) after incubation at 3t °C for 48 h. Yeasts and moulds were counted in potato dextrose agar (PDA) (Oxoid, Basingstoke, Hampshire, UK) added with chloramphenicol (100 mg L^{-1}), after incubation at 25 °C for 48-72 h. Microbiological counts were expressed as log CFU g^{-1} of fennel tissue.

2.2.2 Data elaboration

2.2.2.1 Statistical analysis

The effect on quality parameters of treatment, storage time, and of their interaction was tested by a multifactor ANOVA using StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA), and mean values were separated applying Tukey's Multiple Range Test with significant difference when $p \leq 0.05$.

2.2.2.2 Mathematical modelling for shelf-life estimation

To estimate shelf-life based on MASLT approach for the temporal changes of the quality attributes of fennel samples stored in different storage conditions were arranged in a $Y_T \in X^{NxK}$ matrix to obtain the score and loading matrixes from the accelerated storage tests, where *Y* is the series of experimental data of all measured quality attributes during storage, *T* is the storage temperature, *N* is the number of data point collected during storage and *K* is the number of the quality attributes analyzed. Because the dependent variables had different scales, a previous auto-scaling of the Y_T matrix was conducted to obtain a new Y_{aT} matrix using the method proposed by Pedro and Ferreira (2006).

Then, a Principal component analysis (PCA) was performed on the Y_{aT} matrix on the assumption that the changes of the experimental data over time are inducing the majority of the variability. Assuming that a number of *J* principal components were evaluated as significant for describing the variability of experimental data, a

number of *J* kinetic plots describing the PC scores changes as a function of time were obtained by separating the scores matrix (S) for each T storage condition. Furthermore, the loading matrix (L) was used to map the dependent variables on PC space.

After identifying the PC that showed more relation with time, the changes in scores of this PC were fitted using different kinetic.

Finally, the shelf life of samples of fresh-cut lettuce was estimated by calculating the cut-off criteria (tc) that represent the maximum acceptable scores for each time-related PC (Pedro and Ferreira, 2006):

$$tc = x_a * Lm$$

Where x_a is the vector containing the auto-scaled values of the reference limits of each quality attribute that define the threshold of acceptability of the product, while Lm is the loading matrix of the time-related principal component.

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2.3 EFFECT OF HARVEST TIME ON POST-CUTTING QUALITY OF FRESH-CUT FENNEL cv. TIZIANO

2.3.1 Abstract

The maturity stage is a critical factor for the quality at harvest and after fresh-cut processing. The aim of this study was to evaluate the effect of maturity on quality of fennel heads and on their browning susceptibility after cutting. Fennel heads (cv. Tiziano) were harvested at 7 stages of maturity over a period of 21 days, from HT1 (immature) to HT7 (over-mature), with HT3 as the usual commercial maturity stage. The following quality attributes were evaluated: head weight per cent yield after trimming, respiration rate, stem and sheath color on slices, pH, TSS, TA, total phenolics, vitamin C, sugars, and organic acids. In addition, for each HT, fennel slices were stored for 4 days at 5°C in air and then sensorially evaluated for stem and sheath browning and colour of the cut surface. Fennel heads reached the full size at the usual commercial maturity stage. Maturity at harvest significantly affected respiration rate and quality attributes. Respiration rate increased from HT1 to HT4 and then remained almost constant until HT7. A gradual loss of green color occurred from HT1 to HT7, in both stems and sheaths. Sugars as well as organic acids reached highest values at HT3 and then decreased until the over-mature stage. Total phenol and vitamin C contents showed similar trends, with lower values at HT1 compared to HTs 2, 3 and 4, and then starting to decrease after HT5. Regardless of the HT, after 4 days at 5 °C in air fresh-cut fennels turned brown on the cut-surfaces of stems and on sheaths; however the lower total color variation

was observed in samples harvested at HT2. Results showed that there is a very restricted range of time to harvest fennels in order to have a good quality fresh-cut product. Harvesting fennels heads at the commercial maturity stage ensures high nutritional values and good sugar content along with the highest process yield (since fennels reached the full size). However a slight anticipation of the harvest time could reduce the occurrence of browning in both stem and sheath cut-surfaces of fennel slices during post-cutting storage.

2.3.2 Objective

The aim of this study was to evaluate the effect of maturity at harvest on quality attributes of fennel, also in relation to browning susceptibility when processed as fresh-cut product.

2.3.3 Experimental setup

Fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Tiziano*) was cultivated under commercial conditions in Puglia, Italy. Seedlings were transplanted in an open field at 22 cm distance on the row and at 75cm between rows on September 18, 2014. Fennel plants were manually harvested, following a randomized block design, at different dates corresponding at different maturity stages (harvest time HT) from immature (HT1) to over-mature (HT7), with HT3 as the typical commercial maturity stage. More in details harvests were performed after 133 (HT1), 137 (HT2), 140 (HT3), 144 (HT4), 147 (HT5), 151 (HT6), and 154 (HT7) days from seedlings transplantation. The harvest procedures consisted in

the cutting of the fennel base from the roots; then the stalks were excised using a sharp stainless steel knife. Immediately after harvest, fennel heads were transported to the Postharvest laboratory of the University of Foggia where they were stored at 5° C until processing. For each harvest time, 25 fennel heads were used. Each head was weighed before and after trimming operations in order to determine the weight of the raw material and the percentage of yield for processing. Trimming operations consisted in the cutting of the stalks at the upper base of the fennel heads and in the elimination of outer, more fibrous, leaves. Fennel heads were then washed in tap water, dried and randomly divided in 8 batches of 3 fennel heads each. Three batches were used as replicates for the determination of respiration activity. The remaining 5 batches were individually processed as a replicate. Each fennel head was cut into 8 slices of approximately 1 cm thickness by cutting perpendicular to the longitudinal axis with a sharp knife, with about 24 slices obtained from each replicate. Twelve slices were immediately used for the following determinations:

- stem and sheath color;
- total soluble solid (TSS);
- pH;
- titratable acidity (TA);
- total phenols content;
- vitamin C (total, L-ascorbic and L-dehydroascorbic acid);
- sugars and organic acids.

The remaining 12 slices were placed in macro-perforated polyethylene clam-packs (119 x 189 x 90 mm; capacity 500 g; CL1/90 Carton Pack®), and stored at 5 °C for

4 days. After storage color was measured using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) and then slices were scored for browning by a trained panel of 5 members using a 1 to 5 scale, where 1= absence of browning, 3= slight browning, 5= complete browning.

2.3.4 Results and discussion

Table 2.3.4.1 shows the effect of harvest times on quality attributes of fennels cv. *Tiziano*. In addition stem and sheath browning scores and the total color variation (ΔE) of fennel slices after 4 day of storage at 5 °C was reported. Almost all the parameters analysed were significantly affected by time of harvest, except the yield and titratable acidity.

Table 2.3.4.1 Effect of harvest time on quality parameters of fresh-cut fennels at harvest and after 4 days of post-cutting storage at 5 °C. Data of respiration rate are mean values of 3 replicates; raw material weight and yield were calculated as mean values of 25 fennels for each harvest time; others data are mean values of 5 replicates for each harvest time.

Parameters	HT 1	HT 2	HT 3	HT 4	HT 5	HR 6	HT 7
At harvest							
Physiological attributes							
Respiration rate (mL CO_2 kg^{-1} h^{-1})	2.8 c	3.4 bc	3.0 c	5.6 a	4.2 abc	4.8 ab	3.8 bc
Physical attributes							
Raw material weight (g)	582.4 b	579.4 b	668.9 a	695.7 a	687.6 a	669.7 a	722.1 a
Yield %	36.6 ns	40.5 ns	34.5 ns	35.4 ns	36.1 ns	39.6 ns	40.5 ns
Stem color							
L*	69.9 ab	68.8 b	69.4 ab	71.9 a	68.9 b	69.6 ab	68.2 b
a*	-1.7 b	-1.6 b	-1.7 b	-1.5 b	-0.7 a	-0.5 a	-0.5 a
b*	12.6 b	13.8 ab	13.8 ab	12.4 b	15.5 a	14.5 ab	14.6 ab
Chroma	12.7 ab	13.9 ab	13.9 ab	12.5 b	15.5 a	14.5 ab	14.6 ab
Hue angle	98.1 a	96.7 a	97.1 a	97.1 a	92.5 b	92.2 b	92.1 b
Sheath color							
L*	74.4 b	74.0 b	76.5 ab	77.9 a	77.3 a	77.2 a	77.7 a
a*	-2.3 c	-2.0 bc	-1.9 bc	-1.7 b	-1.0 a	-0.9 a	-1.0 a
b*	12.4 a	12.5 a	11.8 ab	11.8 ab	11.3 ab	10.0 b	12.5 a
Chroma	12.7 a	12.7 a	12.0 a	12.0 a	11.3 ab	10.1 b	12.6 a
Hue angle	100.5 a	99.0 ab	99.2 ab	98.3 b	95.0 c	94.9 c	94.4 c
Chemical attributes							
Total soluble solid (°Brix)	5.9 b	6.5 a	6.6 a	6.4 ab	6.7 a	6.4 ab	6.5 a
pH	6.5 a	6.5 a	6.5 a	6.5 a	6.4 ab	6.3 b	6.4 ab
Titratable acidity (mEq NaOH 100 g^{-1})	1.7 ns	1.8 ns	1.7 ns	1.7 ns	1.9 ns	1.8 ns	1.8 ns
Total phenol content (mg GAE 100 g^{-1} fw)	15.3 c	21.2 ab	22.7 a	24.2 a	16.6 bc	15.7 c	15.7 c
Ascorbic acid (mg 100 g^{-1} fw)	7.8 c	15.2 a	15.0 a	11.9 b	8.9 c	9.0 c	9.1 bc
L-dehydroascorbic acid (mg 100 g^{-1} fw)	4.0 b	5.4 a	5.1 ab	5.3 a	4.9 ab	5.0 ab	4.9 ab
Vitamin C (mg 100 g^{-1} fw)	11.9 d	20.6 a	20.1 ab	17.2 bc	13.8 d	14.1 cd	14.0 cd
Sugars (g 100g^{-1} fw)							
Fructose	1.6 b	2.1 a	2.4 a	1.4 bc	1.2 bc	1.1 c	1.0 c
Glucose	1.0 bc	1.3 ab	1.6 a	1.0 bc	0.9 bc	0.8 c	0.9 bc
Sucrose	1.3 a	1.4 a	1.7 a	0.7 b	0.7 b	0.7 b	0.4 b
Total sugars	3.9 c	4.7 b	5.8 a	3.1 cd	2.8 d	2.6 d	2.2 d
Organic acids (mg 100g^{-1} fw)							
Oxalic acid	459.6 ab	469.1 ab	621.0 a	462.7 ab	324.7 bc	193.5 c	162.0 c
Citric acid	20.2 b	27.1 b	81.8 a	13.5 b	12.8 b	12.1 b	9.9 b
Tartaric acid	2.2 b	4.5 b	17.9 a	5.7 b	6.0 b	6.3 b	7.5 b
Malic acid	522.4 b	647.5 ab	965.2 a	560.8 b	479.1 b	391.8 b	283.6 b
Quinic acid	nd	nd	nd	nd	nd	11.1 a	14.2 a
Succinic acid	395.7 b	431.0 b	870.9 a	354.0 b	325.9 b	302.8 b	279.1 b
Fumaric acid	34.2 ab	23.6 bc	56.3 a	35.9 ab	25.8 bc	16.4 bc	8.8 c
Total organic acids	1434.3 bc	1619.2 b	2613.2 a	1432.7 bc	1174.3 bcd	934.1 cd	765.1 c
After 4 days at 5 °C							
Sensorial attributes							
Stem browning	3.4 a	2.5 b	3.9 a	3.1 ab	3.3 ab	3.3 ab	3.0 ab
Sheath browning	2.4 b	2.0 b	3.4 ab	2.7 ab	3.9 a	2.9 ab	3.2 ab
Physical attributes							
ΔE stem	10.0 a	6.4 b	9.5 a	7.5 ab	9.1 ab	7.2 ab	8.5 ab
ΔE sheath	8.0 ab	6.2 b	10.0 a	8.6 ab	10.2 a	6.0 b	8.0 ab

Different letters indicate statistical differences within harvest times, according to the Tukey's test ($p \leq 0.05$).

At harvest, the weight of fennel heads ranged between 380 and 930 g with some significant differences observed among HTs: the weight of raw material significantly increased at HT3 and then remain substantially constant until HT7. After trimming operations the weight of fennels was proportional to that of the initial raw material (data not showed), and no significant changes in percentage of yield were observed. Average yield was 38%. Changes in the size of any fruit or vegetables crop while growing is frequently used to determine harvest maturity and quality; it is one of the oldest methods of maturity determination. Size increases as a fresh produce approaches toward maturity (Barman et al., 2015). For fresh consumption, growers usually harvest fennel when the heads reach the full size and according to this indication, in the present experiment fennels were ready to be harvested for consumption at HT3. As shown in Table 2.3.4.1, respiration rate significantly changed during plant development: it was lower in the first three HTs (averagely 3.1 ± 0.3 mL CO_2 kg^{-1} h^{-1}) compared to HT4 (5.6 mL CO_2 kg^{-1} h^{-1}); afterward the respiration remained almost constant until HT6, and then decreased again at HT7 (3.8 mL CO_2 kg^{-1} h^{-1}). Changes in respiration rate in fennel heads during development could be related to the different metabolic activity of the sheaths that surround the stem. The inner sheathes are younger and probably have higher respiration rate compared to the external ones. The younger internal sheathes in fact can be considered a meristematic tissue that have higher rate of respiration than older parts (Saltveit, 2002). In the early stage of fennel head development, the younger tissue of the internal sheaths is proportionally lower compared to the older

external sheathes, therefore is possible that its contribution to the rate of respiration was lower and as results, the overall respiration rate is lower than in later fully developed stages.

All the color parameters were significantly affected by harvest date as shown in Table 2.3.4.1. The a* values remained always negative, however a gradual loss of green component was observed from HT1 to HT7, in both stems and sheathes (Figure 2.3.4.1). In particular in the stems a* values were significantly lower (between -1.7 and -1.5) until HT4 compared to HT5, HT6 and HT7. In fennel sheathes the loss of green color occurred more gradually in the first four HTs and significant differences were observed between HT1 and HT4. After HT4 a* values significantly increased, from -1.7 to -1.0, and no differences were observed among HT5, HT6 and HT7. Same trend could be observed in hue angle in both stem and sheath, with a decrease from HT1 to HT7 (Figure 2.3.4.1). In particular hue angle values decreased from 98.1° to 92.1° in the stems and from 100.5° to 94.4° in the sheaths, describing a changes in color from light green to light yellow. The changes observed in a* and hue angle values suggested that there was a degradation of chlorophyll during the completion of the growth of the fennel head. Maunders et al. (1983) reported that during the senescence of the plant tissue the structure of the cytoplasm and chloroplasts is damaged, therefore pigments, such as chlorophyll, are accessible to the attack of acids and enzyme of cellular degradation, favored by the presence of oxygen. Therefore we suggest that the changes observed in a* and hue angle values were probably due to the degradation of chlorophyll during plant development. Regardless to HTs, the green component on the sheath cut-surface

(more negative values) was greater than that in the stem parts (less negative values). This can be attributed to the chlorophyll pigments located in the xylem vessels that pass through the sheaths, leading to a more accentuated green appearance.

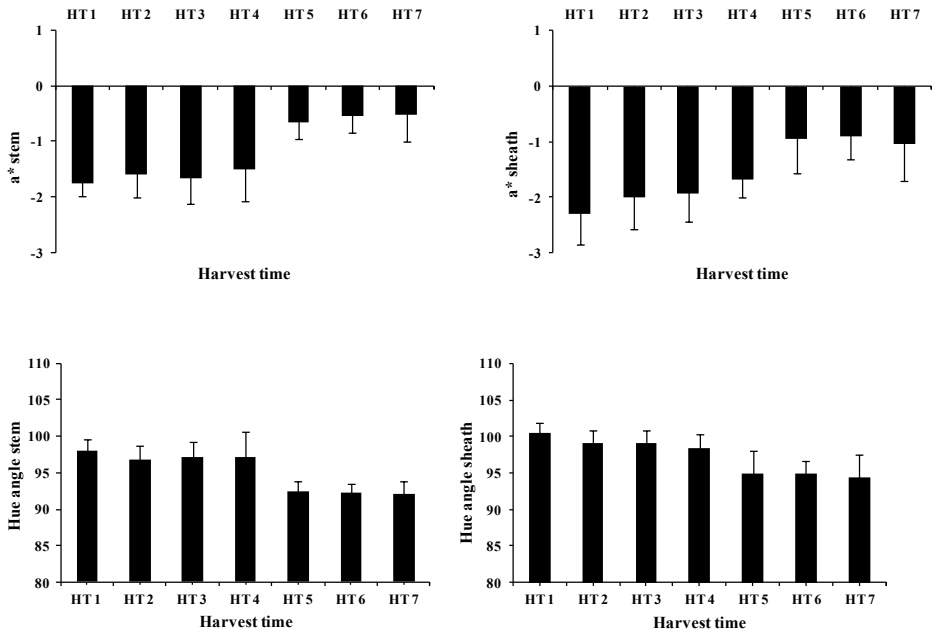

Figure 2.3.4.1 Changes in a* value and hue angle in fennel stems and sheathes at different harvest time. Values are mean of five replicates for each harvest time ± STD.

The L* value did not follow a clear trend during plant development (Table 2.3.4.1): in the stems a higher value was observed at HT4 compared to HT2, HT5 and HT7. The increase in lightness from HT2 to HT4 was probably due to the whitening that occurred from immature to mature stage, as also observed in the sheathes. When fennel heads become over-mature, the luminosity decreased in the stems but not in

the sheathes. Even if no previous studies are available on changes in color of fennels at different maturity stages, experiment conducted on fresh-cut fennels reported afterwards in this dissertation (Experiments 2.5 and 2.6), revealed that stem is much more susceptible than sheath to color changes during storage. Comparing L* values of the external leaves and of the butt end zone of whole fennels during cold storage, Escalona et al. (2004) also reported that a significant decrease in lightness occurred on the butt end zone but not on the external leaves. Therefore a similar behavior in color changes on stems and sheathes seems to occur during final part of development, as in postharvest life of fennel. When fennel is minimally processed changes in color are due to the cutting operations that is assumed to cause disruption of compartmentalization, allowing substrates and enzyme (oxidase) to come into contact (Brecht, 1995) leading to browning. Similar damages in cell structure occur during senescence of the plant and can lead to enzyme-substrate contact, causing color changes. Significant differences in b* value in the stem were observed only comparing HT5 (highest b* value) with HT1 and HT4 (lowest b* values). In fennel sheath color a general decrease of yellow component during development was observed: b* values were highest in the immature stages and gradually decreased during harvest times until the lowest b* value at HT6 (10.0). The increase in b* value at last harvest date (HT7) could be an indicator of a damage as consequence of the plant development. The chromaticity was well described by changes in b* in both stems and sheaths, since the contribution of a* values the calculation of chroma is very low.

After 4 days of cold storage, panelists evaluated the occurrence of browning both on the stems and on the sheaths cut-surfaces, regardless of the HT. The highest stem browning score was observed in fennel at HT3 (score 3.9) and HT1 (score 3.4), while fennel harvested at HT2 resulted less browned on stem as on the sheathes. Sheath cut-surface of fennel heads harvested in the early stage of maturation (HT1 and HT2) resulted less browned (scores 2.4 and 2.0 respectively) compared to that of HT5 (score 3.9) (Table 2.3.4.1). Sensorial data are confirmed by color evaluation of the stems, that also underline a higher stem color variation in fennel harvested at HT1 and HT3, while HT2 showed the lowest ΔE. According to sensorial score, fennel sheathes of HT2 also had the lowest ΔE while the highest value was observed at HT5. In addition color evaluation underline significant difference between HT3 and HT6 that were not perceived by panelists (Table 2.3.4.1).

Almost all chemical attributes of fennels at harvest varied significantly during plant development. Total soluble solid (TSS) were significantly lower at HT1 (5.9 ± 0.1), then increased at HT2 (6.5 ± 0.2) and kept relatively constant up to HT7 (6.5 ± 0.1). Slight variations in pH were observed among HTs: values ranged from 6.3 to 6.5 and significant differences were between HT7 compared with the first four HTs. No changes in titratable acidity were observed during plant development. These results are partially in contrast with data from HPLC analysis of sugars and organic acids. As for TSS, sugars significantly increased from HT1 to HT2 however while the TSS remained constant until HT7, sugars reached the higher content at HT4 and then dramatically decreased (Figure 2.3.4.2).

The increase in TSS as well as in sugars in the first HTs is expected since during plant development sugars are accumulated, whereas the decrease starting from HT4 was probably due to their conversion into more complex non-soluble storage carbohydrates , not detected by a refractometer.

The low correlation between TSS and total sugars content was probably related to the fact that soluble solids measured by a refractometer include not only sugars, but also organic acids (including ascorbic acid), soluble pectins, and phenolic compounds (Kader, 2008). In the present experiment, most of these parameters decreased during plant development, however the higher values of TSS in the last HTs could be related to the presence of soluble pectins that are well represented in fennel (source: compositional database from CREA, Italy).

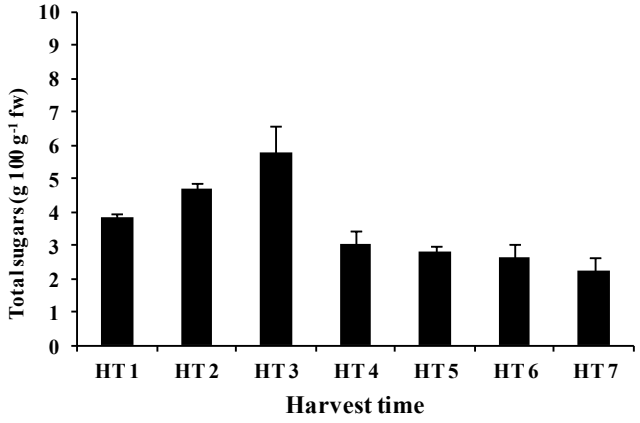

Figure 2.3.4.2 Content of total sugars in fennel at different harvest time. Values are mean of five replicates for each harvest time ± STD.

Similar to sugars, also organic acids significantly increased from immature (HT1) to mature (HT3) stage, when they reached the highest content, and then decreased (Figure 2.3.4.3). Changes in organic acids in the last HTs are probably correlated to changes in respiration rate. During the process of respiration in fact, mainly carbohydrates but also organic acids are broken down to their constituent parts to produce energy to run cellular processes, thus keeping the cells alive (Saltveit, 2002; Silva, 2008). Therefore, the decrease in organic acids after HT4 may be occurred due to an increase in the energy demand of the plant, as evidenced by the increase in respiratory rate at HT4.

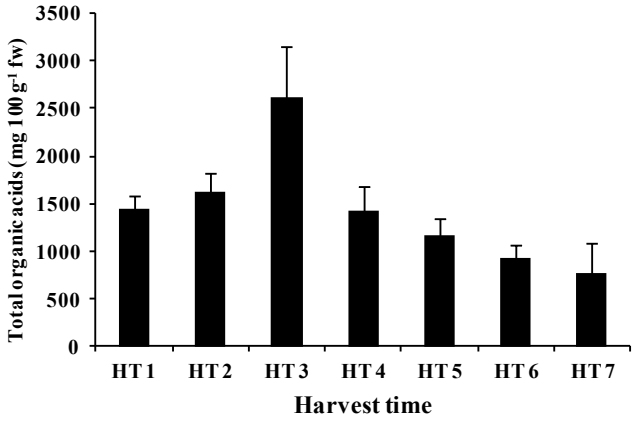

Figure 2.3.4.3 Content of total organic acids in fennel at different harvest time. Values are mean of five replicates for each harvest time ± STD.

Among sugars, fructose was the most abundant representing about 40-45 % of the total sugars at each HT, while, on the other hand, the ratio sucrose over glucose decreased during final development of the fennel head. In particular until HT3 the

percentage of sucrose was higher than that of glucose while, starting from HT4, the sucrose percentage decreased while that of glucose increased (Table 2.3.4.1). This occurred probably because sucrose was hydrolyzed in their monosaccharide's constituents. Present results are in contrast with Barros et al. (2010) that reported as glucose was the most abundant sugar in fennels, regardless the different parts of the plant analyzed. However, according with these authors, the content of sucrose decreased during plant development since they found a loss of sucrose during the development from shoots to stems. No further references are available on changes in sugars and organic acids in fennel during developmental stages. Phan et al. (1973) studied changes in sugars and organic acid in carrot roots during growth, founding that there is a sugars synthesis or accumulation, or both, and the content increased steadily to reach a plateau about 3 months after seeding, while the higher amount of organic acids were reached later than sugars but, differently from them, organic acid slightly decrease after a peak. In the present experiment the 'biochemical maturity', considered as the moment with highest sugar and organic acid contents, was reached when fennel heads completed growth, as expressed by weight of raw fennels at harvest (Table 2.3.4.1). Therefore the 'biochemical maturity' at HT3 corresponds with 'horticultural maturity' as defined by Watada et al. (1984) as "the stage of development when plant or plant part possesses the prerequisites for utilization by consumers for a particular purpose". Regarding organic acids, even if the relative abundance changed during plant development, considering the mean values at different HTs, malic acid was always the most abundant (550 mg $100g^{-1}$ fw) followed by succinic (423 mg 100 g^{-1} fw), oxalic (385

mg 100 g^{-1} fw), fumaric (29 mg 100 g^{-1} fw), citric (25 mg 100 g^{-1} fw) tartaric (7 mg 100 g^{-1} fw) and quinic acid (3.6 mg 100 g^{-1} fw). It is noteworthy that, despite the general trend to decrease of organic acids from HT4, detectable amount of quinic acid was observed only in the last harvest times (HT6 and HT7). Quinic acid is an efficient precursor of aromatic amino acids: in plants it can be converted in shikimic acid which can be further metabolized to aromatic biosynthesis (Minamikawa, 1976). In fact shikimic acid is an intermediate compound in the pathway for the biosynthesis of L-phenylalanine that is utilized in the phenylpropanoid metabolism to synthesize phenolic compounds (Cisneros-Zevallos et al., 2014). The accumulation of quinic acid could be linked to the lower levels of phenolics: it is possible in fact that during maturation quinic acid was rapidly converted in shikimic acid and then in phenolics via L-phenylalanine. As consequence quinic acid was not detected in immature and mature stages but the phenolic content increased, reaching the highest value of 24.2 mg GAE 100 g^{-1} fw at HT4. (Table 2.3.4.1). Afterwards, when phenolic content decrease in the late stages of development, detectable amounts of quinic acid could be observed. Regardless of the possible correlation between quinic acid and phenolic compounds, the latter showed lower values at HT1 compared to HTs 2, 3 and 4, when phenolics reached the maximum amounts, and then starting to decrease after HT5 (Figure 2.3.4.4). In accordance with Tiwari and Cummins (2013), the physiological maturity plays a key factor in influencing the level of phytochemicals. There are little information about phenolic content evolution during growth in vegetables and results are controversial. For instance, similarly to the results on fennels, during “maturation” of ‘Cool Guard’ lettuce

harvested at three stages of maturity phenolics were higher in the immature and mature stages (133 and 134 μg g^{-1} respectively) than in the over-mature stage (114 μg g^{-1}) (Couture et al., 1993). Also Chutichudet et al. (2011) reported that for lettuce cv. 'Grand Rapids' phenolics reached the maximal content at the early developmental time, and then decreased dramatically to increase again at the late-harvesting. Pandjaitan et al. (2005) found a highest level of total phenolics as well as total flavonoids in middle leaves of spinach plants, suggesting that these compounds were synthesized in leaves at early stages of maturity, decreasing during the final maturity.

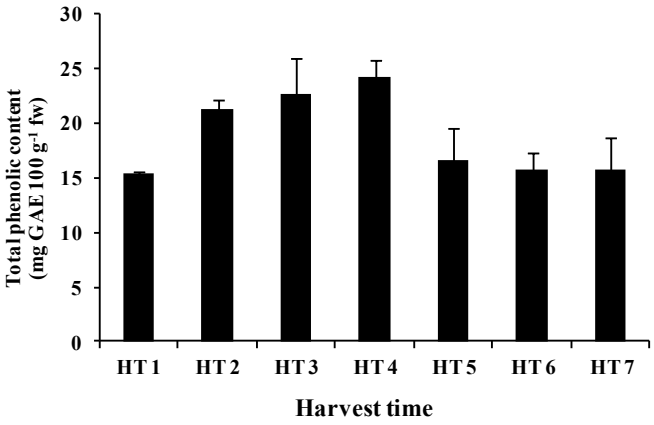

Figure 2.3.4.4 Content of total phenolics in fennel at different harvest time. Values are mean values of five replicates for each harvest time ± STD.

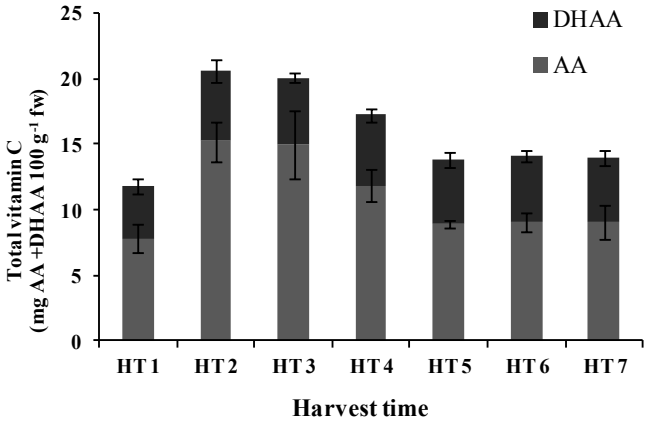

Figure 2.3.4.5 Content of total phenolics and vitamin C in fennel at different harvest time. Values are mean of five replicates for each harvest time ± STD.

As for sugars, organic acids, and phenolics, also total vitamin C showed a similar pattern during plant development: it significantly increased until HT3 and then decreased. AA increased significantly from HT1 to HT2 and then decreased after HT3, whereas DHAA, after the increase from HT1 to HT2, remained almost constant until the over-mature stage (Table 2.3.4.1). Thus, the increase in total vitamin C from HT1 to HT2 was mainly due to the increase of ascorbic acid (from 7.8 to 15.2 mg 100 g^{-1} fw) but also to that of DHAA, which significantly increased from HT1 (4 mg 100 g^{-1} fw) to HT2 (5.4 mg 100 g^{-1} fw), while after HT3, changes in total vitamin C were due to changes in ascorbic acid (Figure 2.3.4.5). The increase of AA during HTs was expected since it was previously reported that ascorbic acid is accumulated in plant tissues undergoing active growth and development (Lee and Kader, 2000). In the late stage of development, the decrease in AA could depended of its oxidation in DHAA by the enzyme ascorbate oxidase

(AAO) that has been proposed to be the major enzyme responsible for enzymatic degradation of AA (Mehlhorn, 1990; Saari et al., 1995). Ascorbate oxidase is associated with rapidly growing regions in the plant (Lee and Kader, 2000); therefore this enzyme was probably largely present in the late HTs, when the younger sheathes represented the higher portion of the total fennel sheathes, catalyzing the degradation of AA in DHAA. However, DHAA can be further degraded to diketogluconic acid (Parviainen and Nyyssonen, 1992), and this could be the reason why in the present experiment the decrease in AA in the late HTs was not accompanied with an increase in DHAA. In accordance with the present data, Weston et al. (1997) reported in snap beans and other green vegetables that ascorbic acid tends to increase with maturation and decrease with advanced maturation. A similar behavior of vitamin C during various stages of maturity was observed also by Yahia et al. (2001) on tomato and bell peppers where ascorbic acid increased to reach a maximum level and then decreased.

To better clarify the influence of harvest times on the main quality parameters of fennels, a principal component analysis (PCA) was performed (Figure 2.3.4.6). The different harvest times were separated on the base of the main quality parameters analyzed using the first and the second PC factors which retained 86.6% of the total variance. The first PC factor explained 65.2% of total variance and separated the first 4 harvest times (HT1, HT2, HT3 and HT4), placed on the right hand side of the PC1, from the late harvests (HT5, HT6 and HT7) there are grouped close together on the left hand side. The separation of HTs on the PC1 was mainly related to the content of organic acid, sugars, total phenolic and vitamin C contents, as well as to

changes in hue angle in both fennel stems and sheathes that were higher in the HTs placed on the right hand side of the PC1. Late harvest times (HT5, HT6 and HT7) were therefore characterized by the lower organic acid, sugar, phenolic and vitamin C content, and by a higher respiration rate. HT1 and HT4 are well separated on the PC2 that explained 21.1% of the total variance: in this case, differences between these HTs were mainly related to phenolic and vitamin C contents and to the respiration rate that were higher in HT4 compare to HT1. As suggested by the arrows in the PC1-PC2 plane, all the parameters analyzed gradually changed during plant development: respiration rate increased, sugars and organic acid were accumulated in the HT2 and HT3 while phenolics and vitamin C increased slightly later, between HT3 and HT4. From the color point of view the position of hue angle in the PC1-PC2 plane indicates that hue of fennel stems and sheathes decreased from immature to over-mature stages.

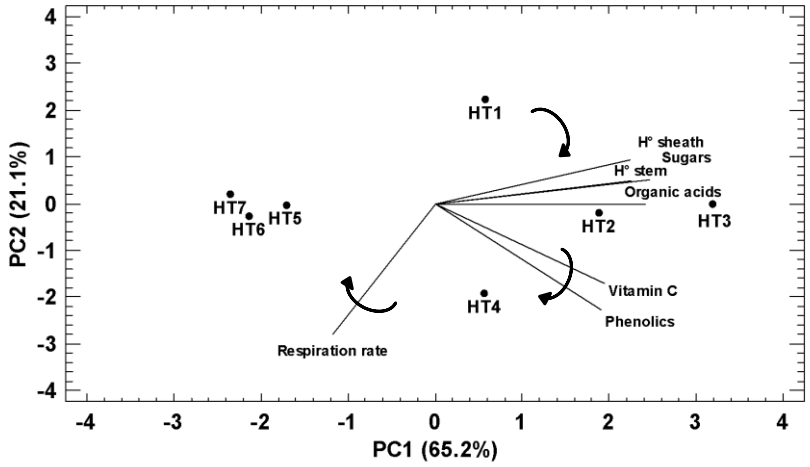

Figure 2.3.4.6 Principal Component Analysis (PCA) of quality parameters of fennel cv. Tiziano at different harvest time.

2.3.5 Conclusion

Harvesting fennels heads at the commercial maturity stage, beside ensuring the achievement of the maximum size, provided heads with high nutritional (highest values of phenolic compounds and vitamin C), and organoleptic (good sugar content) properties. As for fennel suitability to be processed as fresh-cut product a slight anticipation of the harvest time could reduce the occurrence of browning in both stem and sheath part of the slices during post-cutting storage.

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2.4 THE USE OF HYPERSPECTRAL IMAGING TO PREDICT THE DISTRUBUTION OF INTERNAL CONSTITUENTS AND TO CLASSIFY EDIBLE FENNEL HEADS BASED ON THE HARVEST TIME

2.4.1 Abstract

The objective of this study was to use hyperspectral imaging to predict the internal content of different quality attributes such as soluble solids, individual sugars and organic acids, phenols, and antioxidant activity of fennel heads also in relation to different sheat layers and harvest times. Thirthytwo fennel heads were collected during 7 different harvests over a period of 3 weeks. For each fennel 2 images of the perpendicular section (cut in the middle of the head) were acquired with a Hyperspectral scanner by using 2 spectrographs in the VIS NIR(400 to 1000 nm) and in the NIR region (900-1700 nm). For prediction purposes, 5 leaves (including the stem) were individuated from the external to the internal part and grinded to get the tissue puree to be used for chemical extraction, obtaining a total of 160 samples. In the same way from hyperspectral images more regions of interest (ROI) extracted for each corresponding leaf were averaged obtaining a total of 160 spectra. Reference content values were then used to build the Partial Least Square Regression (PLSR) for each of the 2 spectral datasets. After removing 20 samples for which one or more reference analysis could not be carried out, and 4 outlier spectra, over 140, a calibration set of 105 samples and a validation set of 31 samples was used to develop the models. Among the predicted parameters only

phenols, total soluble solids, and antioxidant activity could be predicted with satisfactory accuracy whereas the other compounds were predicted with very low performances. For all these 3 parameters VIS-NIRS gave better results than NIR spectra, and this is probably because some information is retained in the color and also because the maximum absorbance value for the standards of the reference method, gallic acid (GA) for phenols and Trolox for antioxidant activity, are also registerd at 725 and 515 nm, respectively. Particularly, for soluble solids, after applying Mean centering R^2 of 0.869, 0.807, 0.768 were obtained for calibration, cross validation, and prediction, respectively (RMSEP of 0.5 over a range of values from 4 to 9 °Brix). For antioxidant activity the model gave the same accuracy with R^2 of 0.856, 0.805, 0.745 (RMSEP of 2.76 over a range from 2 to 25 mg of Trolox/100 g) applying smoothing and Mean centering. Also for phenols the best preprocessing technique resulted smoothing and mean center and R^2 obtained were recorded to be 0.809, 0.794 and 0.787, with RMSEP of 3.113 (over a range from 5 to 35 mg Gallic acid equivalent/100g). Moreover it is interesting to observe that soluble solids, phenolics and antioxidant activity increased from the external to the internal leaves, and that this variation can be observed on hyperspectral images by mapping the constituent concentrations. Classification based on time of harvest was done using the PLS-DA by averaging the spectra of all the layers of each fennel. Calibration dataset was pre-processed with MSC (mean), resulting the best pretreatment. All the classes were distinguished with a non-error rate of 92.29% in calibration and 81.86% in cross validation. It was observed that all the samples in all the classes were correctly classified except a few samples of classes 2 and 5 for

calibration and 2, 3 and 5 in case of cross validation. To improve classification performance, similar samples were merged in 4 new classes (harvest 1, harvest 2 and 3, harvest 4, and harvest 5, 6 and 7), resulting in a significant rise of non error rate. In conclusion results of this works show the potentiality of hyperspectral imaging in the VIS-NIR spectral range to predict internal content of soluble solids, phenols and antioxidant activity and to classify fennel heads according to the harvest time.

2.4.2 Objective

The objective of this study was to use hyperspectral imaging to predict the internal content of different quality attributes such as soluble solids, individual sugars and organic acids, phenols, and antioxidant activity, also in relation to different sheath layers of fennel heads in order to have a spatial distribution of these constituents. Moreover a second objective was to apply a classification algorithm to discriminate among fennel heads from different harvest times.

2.4.3 Materials and methods

2.4.3.1 Experimental design and spectral acquisition

Thirty-two fennel heads were collected during 7 different harvests over a period of 3 weeks, in order to enlarge the span of the variation interval of each individual constituent. For each fennel 2 images of the perpendicular section (cut in the middle of the head) were acquired using a Hyperspectral scanner (version 1.4, DV srl,

Padova, Italy) with 2 spectrographs, one in the VIS-NIR range (from 400 to 1000 nm) and the second in the range of 900 to 1700 nm (spectral resolution of 5 nm and spatial resolution 315dpi). For each fennel section 5 leaves or sheath were individuated from the external to the internal part (including the stem). Then these 5 leaves were grinded to get the tissue puree to be used for chemical extraction, obtaining a total of 160 samples. For each sample different extraction procedure allowed to measure total soluble solid content, titratable acidity, antioxidant activity, phenolic content, and sugar and organic acid composition. Over 160 samples, 20 samples were eliminited for some problems in the reference values or one or more quality parameters.

From each corresponding sheath layer of a single fennel, three regions of interest (ROIs) were acquired separately from the images of the VIS-NIR range and the NIR range, using the image cropping tool in the PLS toolbox. For prediction purpose, the mean spectra of the ROIs were averaged, obtaining one spectra per each fennel layer for a total of 140 spectra, as the corresponding number of samples and reference measures. For classification purpose, for all 32 fennel heads, the spectra of different layers were averaged, obtaining one spectra for each fennel, for a total of 32 spectra.

2.4.3.2 Principal component analysis (PCA)

To detect outliers a PCA was performed separately on the spectra of the VIS-NIR and the NIR range. The data acquired using the hyperspectral imaging device were in this way reduced to few variables that account for the system, called Principal

component (Abbott, et al.) which is a linear combination of the original variables. Outliers are the data points the lie away from the normal scattering of the data and may occur due to experimental error or measurement variability. Four spectra were detected as outliers for both VIS-NIR and NIR datasets.

2.4.3.3 Partial least squares regression (PLSR)

PLSR finds the combinations of predictor values that have a greater covariance with the response. PLSR was done using PLS toolbox to achieve calibration models for each quality parameter, by testing the effect of some prior pre-processing such as mean centering, MSC (mean), smoothing and derivatives, on model performance. After removing 4 outliers over 140 spectra, a calibration set of 105 samples and a prediction set of 31 samples were used to develop the models. The calibration models were tested applying the cross validation with 5 splits and 1 sample per blind. For each quality parameter, the prediction ability obtained in calibration and cross validation after pre-processing, was further tested on the external prediction dataset. Moreover for SST the model was applied to map the concentration of an image of one fennel section from the prediction dataset. In this case the model is applied on the average spectra of each pixel and the obtained prediction value is represented by a color referring to a color scale normally ranging from blue (low concentration) to red (high concentration).

The accuracy of calibration depends on the model errors, namely, root mean square error for cross validation (RMSECV) and root mean square error for prediction (RMSEP) used for internal or external validation, respectively. The last value

parameters, defined as follow, gives the average of uncertainty that can be expected for predictions of future samples in the 95% confidence interval.

$$RMSEP = RMSECV = \sqrt{\frac{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2}{n_p}}$$

Where, $\hat{y}_i$ is the predicted value of an attribute in fruit number i; y_i is the measured value of an attribute in fruit number i; n_p is the number of validated cases.

The number of latent variables in the calibration model is typically determined as that which minimizes the RMSECV or RMSEP.

Another useful statistic is the R^2 value. It essentially represents the proportion of explained variance of the response variable in calibration (R_c^2), cross validation (R_{cv}^2) or external prediction (R_P^2) sets.

2.4.3.4 Partial least squares Discriminant analysis (PLS-DA)

The PLS-DA model is a supervised algorithm based on the relation between spectral intensity and sample characteristics; in the present study the X variables represent the spectral variations for each sample and the Y variables the corresponding class. During the calibration process, the PLS-DA method is trained to compute the "membership values", one for each class; the sample is then assigned to one class when the value is above a specific prediction threshold. (Musumarra, et al. 2005, Liu, et al. 2008).

Spectra subjected to the various pre-processing techniques were used to construct the model which, due to the low number of samples, were only evaluated in cross validation (5 splits with 1 sample per blind). Classification model have been

evaluated for sensibility and specificity; the former is the probability that the sample, effectively with the characteristic awaited, is positive to the test; the latter is the probability that the sample, effectively without the characteristic awaited, is negative to the test.

Confusion matrix in which the diagonal objects represent the correctly classified objects can be a good indicator of the classification model performance. It also leads to the development of valuable indices such as non-error rate or classification rate which represents the percentage of the correctly classified samples and is the average of the sensibility calculated over the classes.

2.4.4 Results and discussion

2.4.4.1 Prediction of internal constituents

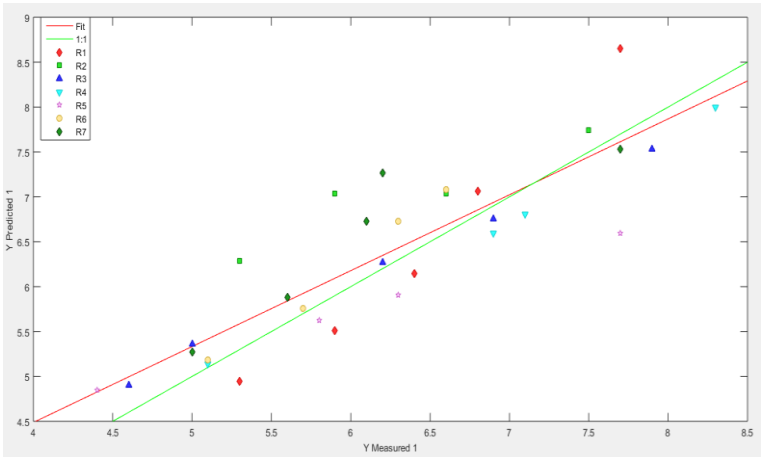
The prediction models were developed in the spectral ranges of 400-1000nm and 900-1700nm. Among many predicted parameters only phenols, total soluble solids, and antioxidant activity could be predicted with satisfactory accuracy considering the lowest values of RMSEC, RMSECV and RMSEP whereas the other compounds were predicted with very low performances.

For all these three parameters, the models gave better results in the VIS-NIR as compared to the NIR spectral range. For this spectral range the effect of preprocessing treatment on the model performance is reported in Table 2.4.4.1.1. For SSC the best model, based on the highest R^2 and lowest errors, was obtained when mean center was applied. The results for this model indicated an R^2 of 0.87

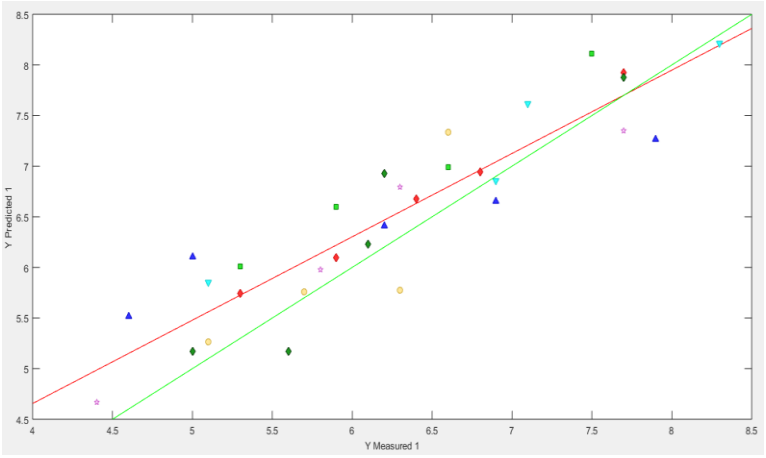
(RMSEC=0.388), 0.81 (RMSECV=0.475) and 0.77 (RMSEP=0.768) for calibration, cross validation and prediction, respectively. Figure 2.4.4.1.1 shows the fitting of Y predicted against the Y measured, for both the NIR and the VIS-NIR regions. It can be observed as for NIR model the prediction curve (in red) and calibration curve (in green) are diverging more than for VIS-NIR (RMSEC=0.497, and RMSEP=0.489).

Table 2.4.4.1.1 Pretreatments effect on the prediction model performance of internal quality attributes of fennel heads.

Parameter	**Preprocessing**	LVs	R_c^2	*RMSEC*	R_{cv}^2	*RMSECV*	R_{pred}^2	*RMSEP*
^{o}Brix	MSC (mean)	4	0.80	0.476	0.76	0.529	0.85	0.470
	Smoothing + MSC (mean)	4	0.76	0.529	0.71	0.578	0.77	0.573
	1st derivative + mean centering	7	0.87	0.383	0.81	0.474	0.74	0.542
	Mean centering	**7**	**0.81**	**0.388**	**0.81**	**0.475**	**0.77**	**0.515**
DPPH	MSC (mean)	5	0.78	2.456	0.73	2.716	0.79	2.423
	Smoothing + MSC (mean)	5	0.67	2.984	0.63	3.194	0.72	3.008
	1st derivative + MSC (mean)	5	0.80	2.334	0.76	2.577	0.79	2.417
	1st derivative + mean centering	**7**	**0.86**	**1.984**	**0.81**	**2.319**	**0.75**	**2.758**
Phenols	MSC (mean)	4	0.82	2.716	0.79	2.885	0.71	3.409
	Smoothing + MSC (mean)	4	0.76	3.078	0.73	3.277	0.63	3.897
	1st derivative + MSC (mean)	3	0.82	2.695	0.81	2.793	0.72	3.423
	2nd derivative + mean centering	**2**	**0.81**	**2.765**	**0.79**	**2.873**	**0.79**	**3.113**
Sucrose	MSC (mean)	2	0.17	0.668	0.12	0.689	0.03	0.376
	Smoothing + MSC (mean)	2	0.17	0.668	0.12	0.689	0.03	0.376
	1st derivative + MSC (mean)	3	0.25	0.634	0.18	0.668	0.42	0.325
	2nd derivative + mean centering	2	0.20	0.657	0.14	0.681	0.14	0.352



a) VIS-NIR range



b) NIR range

Figure 2.4.4.1.1 Y measured vs Y predicted for SSC in the VIS-NIR (a) and NIR range (b).

The most effective wavelengths for the model in the VIS-NIR as evaluated by the VIP score were found to be in the spectral range of 730-920 nm with 2 peaks recorded at 780 nm and 835 nm.

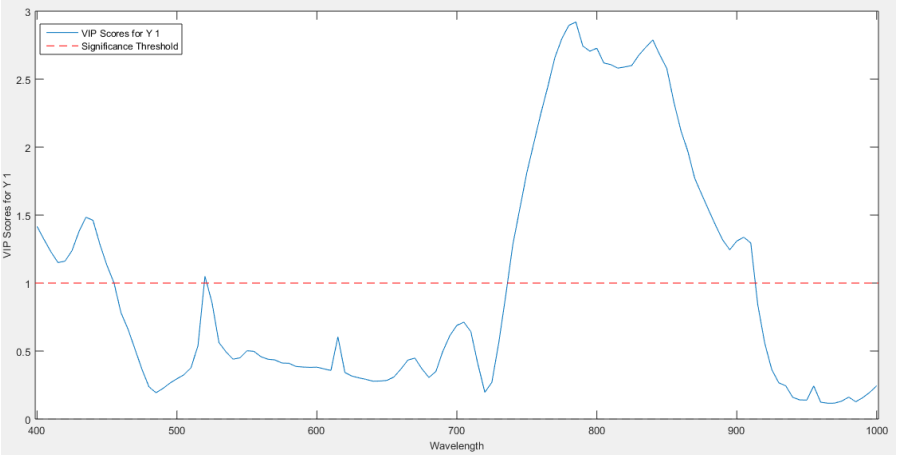
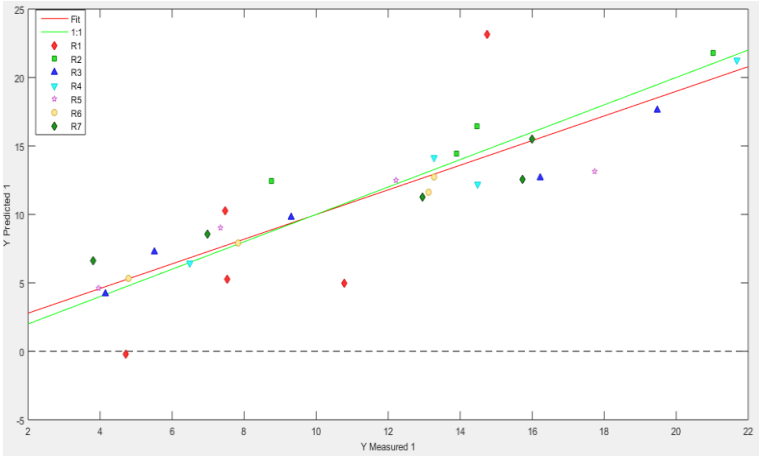


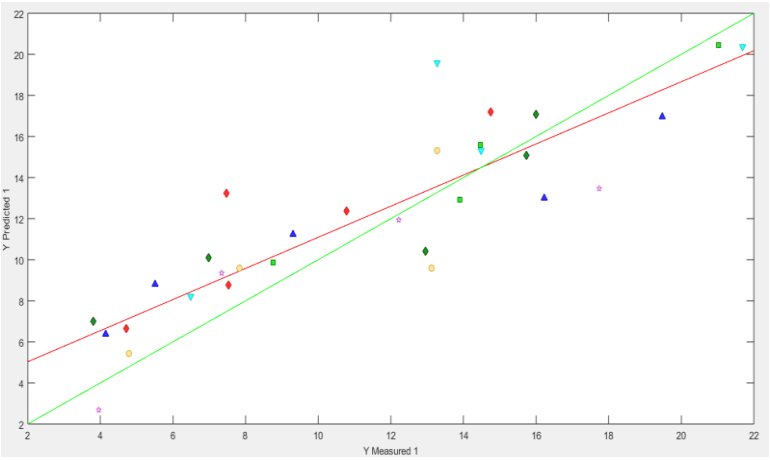
Figure 2.4.4.1.2 Wavelength vs VIP Scores for SSC for the VIS-NIR prediction model.

In case of the antioxidant activity, encouraging results were obtained after pre-processing the data of VIS-NIR using 1st derivative followed by mean centering. The values of R^2 for calibration, cross validation and prediction were 0.86, 0.81 and 0.75, respectively with RMSEC of 1.984, RMSECV of 2.319 and RMSEP of 2.758. On the other hand R^2 of the best model obtained in the NIR range, by applying the same pretreatment, were 0.73, 0.64 and 0.78 for calibration, cross validation and prediction with the values of 2.730 (RMSEC), 3.167 (RMSECV) and 2.564 (RMSEP), having RMESC and RMSECV higher than those achieved for PLS in

visible range. Figure 2.4.4.1.3 shows the prediction data plot of measured values of Y against the predicted values for both spectral ranges.



a) VIS-NIR range



b) NIR range

Figure 2.4.4.1.3 Y measured vs Y predicted for antioxidant activity in the VIS-NIR (a) and NIR range (b).

Looking at the VIP scores 3 peaks were observed at 470, 740 and 880nm, but with first 2 weighting much more than the last one (Figure 2.4.4.1.4).

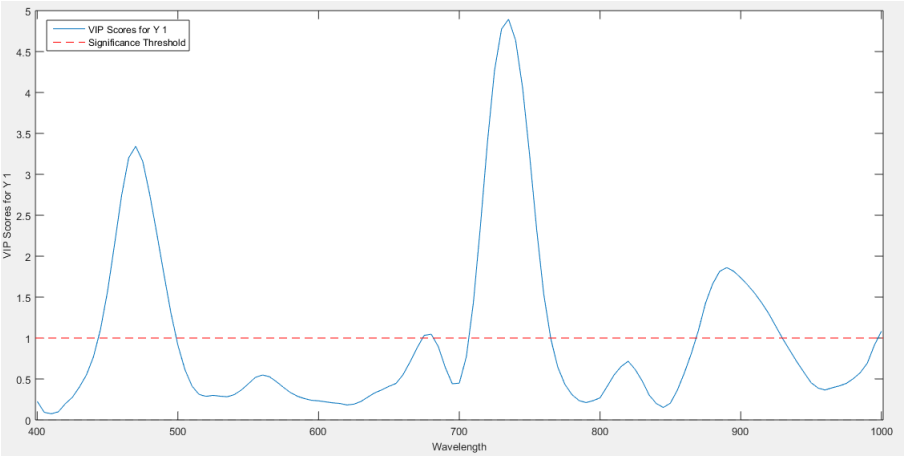
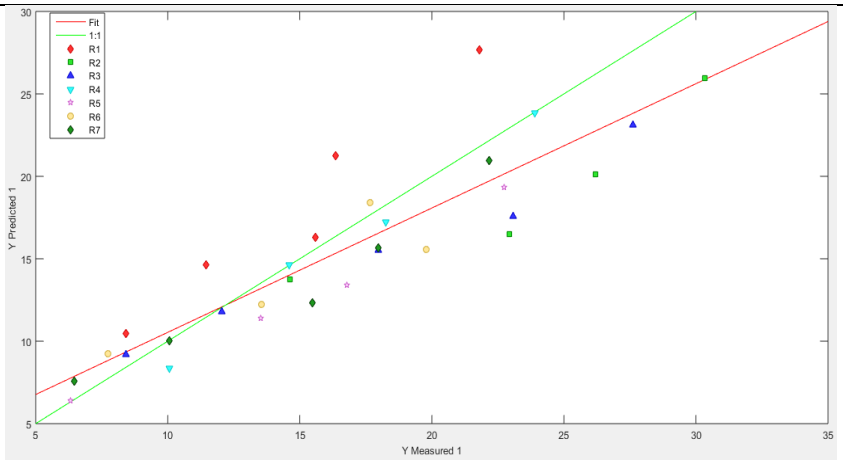
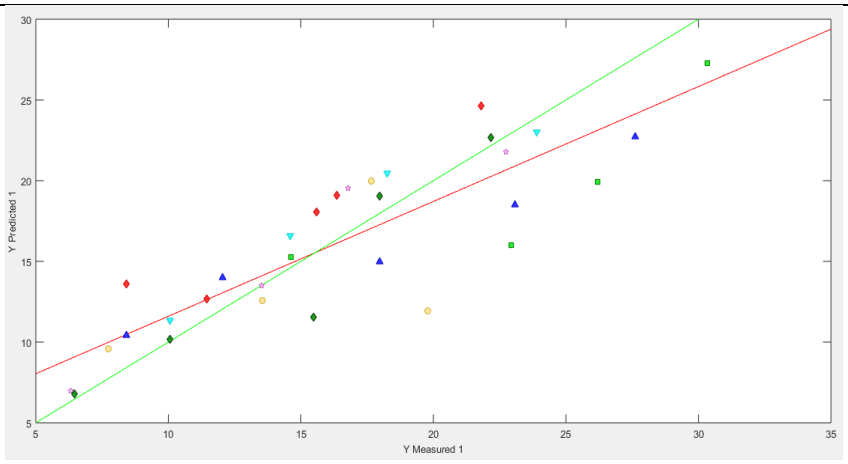


Figure 2.4.4.1.4 Wavelength vs VIP Scores for antioxidant activity for the VIS-NIR prediction model.

For phenols, many preprocessing techniques were used but the best was found to be 2^{nd} derivative followed by mean centering which gave high R^2 values for calibration, cross validation and prediction as 0.81, 0.80 and 0.79, respectively, with RMSEC of 2.765, RMSECV of 2.873 and RMSEP of 3.113. As observed in the case of antioxidant activity the values of the root mean square of calibration, cross validation and prediction were higher in case of NIR models with the best model having R^2 of calibration of 0.73, cross validation of 0.69 and prediction of 0.75. Figure 2.4.4.1.5 shows the plots scores for the Y measured against Y predicted, for both models.



a) VIS-NIR range



b) NIR range

Figure 2.4.4.1.5 Y measured vs Y predicted for Phenols in the VIS-NIR (a) and NIR range (b).

The model mostly depended on the wavelength ranges between 660 to 730 nm, and in lower measure to the region 440-460nm, and 740-770nm with some peaks observed at 450, 500, 710 and 75 0nm.

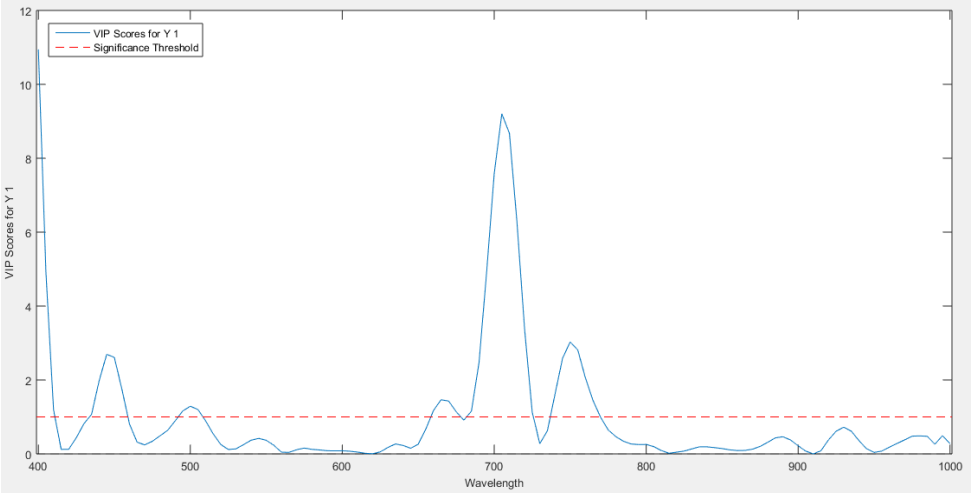


Figure 2.4.4.1.6 Wavelength vs VIP Scores for Y in phenols for the VIS-NIR prediction model.

The results of the prediction of the remaining parameters are not reported, due to the low accuracy. The other parameter measured was sucrose which is an organic sugar and the prediction capability of the models was found to be very low. The best model in the Vis-NIR region preprocessed with smoothing gave for calibration a R^2 value of 0.22, R^2 cross validation of 0.15 and R^2 of prediction of 0.16 with the RMSE values of 0.649, 0.681 and 0.651 for calibration, cross validation and prediction. For the same parameter in the NIR range the data was preprocessed with MSC (mean) to get the regression coefficient values of calibration, cross validation

and prediction of 0.19, 0.12 and 0.39, respectively with the RMSEC of 0.660, 0.694 and 0.352 (Table 2.4.4.1.1).

Finally for all obtained models an image showing the distribution of internal constituents over the fennel sections can be obtained. The distribution map of TSS concentration over the fennel section is shown in Figure 2.4.4.1.7. By this image it can be visually appreciated as TSS increase passing from external to internal leaves and as the xylematic vessels were carrying the sugars to the leaves, showing for each layer the highest concentration.

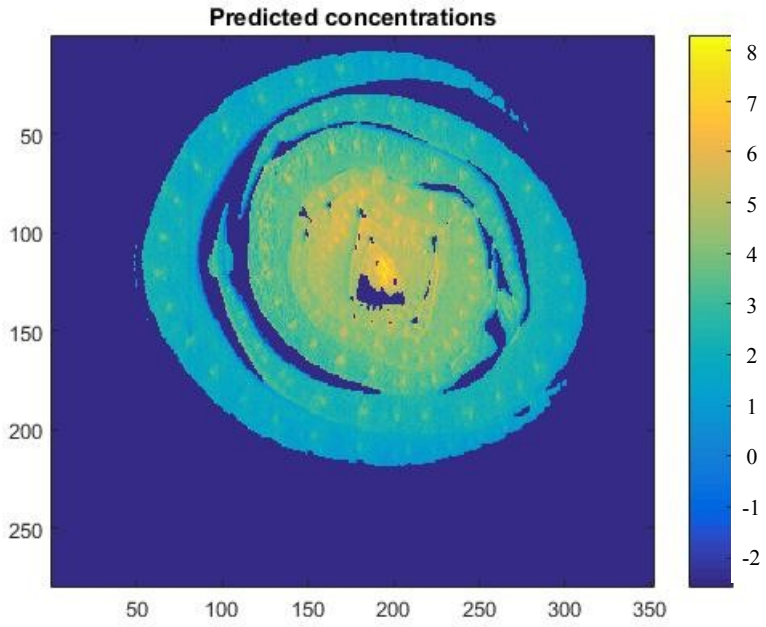


Figure 2.4.4.1.7 Distribution map of TSS concentration over a fennel section.

In the previous studies for the prediction of the same parameters in different fruits similar results were found, as for TSS on strawberry fruits (correlation coefficient for calibration was 0.80 with a SEC of 0.233), obtained on spectra of the same range of 400-1000nm (ElMasry, et al. 2007). Similarly, another study focused on the measurement of the mandarin fruit SSC it was reported that the Vis-NIR spectral region was capable to yield correlation coefficient between the predicted and measured values of 0.94 with a RMSE of 0.33 (Gómez, et al. 2006). In most of the studies in the field of Vis-NIR and NIR spectroscopy the parameters of interest are measurement of SSC, antioxidant activity, phenols and organic sugars (Cozzolino, et al. 2004, Cayuela 2008, Fu, et al. 2015), but very few have been addressed to the study of the fennel.

2.4.4.2Classification by harvest time

Calibration models were developed for the discrimination of fennel heads according to the 7 harvest times, using PLS-DA. The PLS-DA for the classes was achieved by taking the average of the spectra of all the layers of the fennels for all harvest times. The Y variables in this case were the classes, used as 'dummy' variables (Naes, et al. 2002). Table 2 shows the calibration model results (confusion matrix) for the PLS-DA conducted on a dataset of 32 samples. The best pretreatment of the data was MSC (mean) which helped the sensitivity and specificity to get values closer to one in case of calibration and cross validation. Table 2.4.4.2.1 shows the values of the correctly and incorrectly classified samples, along with the depiction of sensitivity and specificity, for each class.

Table 2.4.4.2.1 Confusion table and model parameters of classification of fennel heads from different harvest times obtained applying PLS-DA.

Calibration	*CV*	*Actual Class*							*N*	**Global**	
		H1	***H2***	***H3***	***H4***	***H5***	***H6***	***H7***		**SENS**	**SPEC**
Predicted as Class	***H1***	5	0	0	0	0	0	0	5	100	100
	H2	0	4	0	0	1	0	0	5	80	96
	H3	0	0	5	0	0	0	0	5	100	100
	H4	0	0	0	4	0	0	0	4	100	100
	H5	0	1	0	0	2	0	0	3	66	97
	H6	0	0	0	0	0	5	0	5	100	100
	H7	0	0	0	0	0	0	5	5	100	100
TOTAL									**32**	Non error rate = 92.29	
Cross Validation	***CV***	***Actual Class***							***N***	**Global**	
		H1	***H2***	***H3***	***H4***	***H5***	***H6***	***H7***		**SENS**	**SPEC**
Predicted as Class	***H1***	5	0	0	0	0	0	0	5	100	100
	H2	0	4	0	0	2	0	0	5	80	93
	H3	0	0	3	0	0	0	0	5	60	100
	H4	0	0	0	4	0	0	0	4	100	100
	H5	0	1	2	0	1	0	0	3	33	90
	H6	0	0	0	0	0	5	0	5	100	100
	H7	0	0	0	0	0	0	5	5	100	100
TOTAL									**32**	Non error rate = 81.86	

In the confusion matrix the colored diagonal shows the number of samples correctly classified and N represents the total number of samples in each class. It is evident

that the sensitivity for cross validation of class H5 is very low, and that samples of this class are confused with sample of classes H2 and H3. In all other cases the sensitivity is 100% except for classes H2 (80%) and H5 (66%). Another parameter that can be calculated for the PLS-DA is the "Non Error Rate" that is the average of the sensibility calculated over the classes. The "Non Error Rate" is 92.29% for the calibration and 81.86% in case of cross validation.

Concerning the classes H2 and H5, we find specificity values higher than the sensibility in case of calibration as well as cross validation. To improve classification results, the number of classes may be reduced, may be on the bases of "a-priori" sample knowledge. Classes were therefore reduced based on previous results of an experiment aimed to evaluate the effect of maturity at harvest on quality characteristics and chemical composition of fennel heads (Experiment 2.3). Results of this experiment grouped the same samples in 4 groups, according to their composition and post-cutting performance, resulting in harvest 1 (H1), harvest 2 and 3 (H23), harvest 4 (H4) and harvest 5, 6 and 7 (H567). Using these new 4 classes, a significant increase in the non-error rate was observed for both calibration (94.25%) and cross validation (94.25%).

Table 2.4.4.2.2 Confusion table and model parameters of classification of fennel heads from different harvest times grouped in 4 classes, obtained applying PLS-DA.

Calibration	***CV***	***Actual Class***				***N***	**Global**	
		H1	***H23***	***H4***	***H567***		**SENS**	**SPEC**
Predicted as Class	***H1***	5	0	0	0	5	100	100
	H23	0	10	0	3	10	100	86
	H4	0	0	4	0	4	100	100
	H567	0	0	0	10	13	77	100
TOTAL						**32**	Non error rate = 94.25	
Cross Validation	***CV***	***Actual Class***				***N***	**Global**	
		H1	***H23***	***H4***	***H567***		**SENS**	**SPEC**
Predicted as Class	***H1***	5	0	0	0	5	100	100
	H23	0	10	0	3	10	100	86
	H4	0	0	4	0	4	100	100
	H567	0	0	0	10	13	77	100
TOTAL						**32**	Non error rate = 94.25	

Few previous studied have been conducted to classify different fruits and vegetables. In a study on table grapes the authors used Vis/NIR range to discriminate fruits from different harvest times, comparing SIMCA and PLS-DA. PLS-DA proved to be more effective for the classification purpose achieving 100% correct classification on 4 classes over 5 and 94% of the corrected classified samples in the 5th harvest time (Piazzolla, et al. 2013). Another work (Berardi doctoral thesis unpublished) focused on the classification of fresh-cut artichokes

based on harvest times developing models for 4 and 3 classes, achieving the best results in sensitivity and specificity when only 3 classes were used.

2.4.4 Conclusions

In conclusion results of this works show the potentiality of hyperspectral imaging in the VIS-NIR spectral range to predict internal content of soluble solids, phenols and antioxidant activity of fennel heads. In addition this tool may provide important information about the harvest time of fennel heads, which may be used to determine the optimal harvest time. These results may be interesting for further implementation of non destructive techniques for on-line sorting machines to detect the internal quality and to discriminate among fennel heads of different harvest and maturity. Moreover other factors as the location of origin, the production system, or the variety can be object of further studies in order to increase the amount of information which can be obtained at the same time by one spectral scan.

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2.5 EFFECT OF ANTI-BROWNING SOLUTIONS ON QUALITY OF FRESH-CUT FENNEL cv. ORION DURING STORAGE

2.5.1 Abstract

Fresh-cut fennel is a very perishable crop due to the browning that affects the cut-surface, especially on the stem portion of the slices. The occurrence of browning is the main cause of quality loss and decrease of visual acceptance of this product. In the present experiment the effectiveness of different antibrowning solutions on maintaining quality characteristics of fresh-cut fennel during storage at 5 °C were investigated. Results showed that dipping in solutions of citric acid, ascorbic acid, cysteine, did not result in substantial improvement of the appearance of fresh-cut fennels cut-surfaces compared to water control. Among all applied antibrowning solutions, dipping in 1% citric acid and 0.5% 4-hexylresorcinol produced a more severe browning than control, in both stem and sheath cut-surfaces. Dipping for 2 minute in 0.5% ethanol was effective in preserving visual quality of fresh-cut fennel stored in air for 6 days at 5 °C, significantly reducing the browning in both stem and sheath cut-surface during storage. In addition ethanol is a 'generally recognized as safe' (GRAS) product and did not negatively influence the aroma of fresh-cut fennel. Based on these considerations, the dipping in 0.5% ethanol for 2 min could be a useful pretreatment for extending the shelf-life of fresh-cut fennel.

2.5.2 Objective

The objective of the present experiment was to investigate the effectiveness of different antibrowning solutions on maintaining quality characteristics of fresh-cut fennel during storage at 5 °C.

2.5.3 Experimental setup

Fennel heads (Foeniculum vulgare Mill. subsp. vulgare var. azoricum cv. Orion) were harvested on December 2013 in Puglia (Italy), transported in refrigerated conditions to the Postharvest laboratory of the University of Foggia and kept at 0 °C until processing. After trimming operations, fennel heads were washed in chlorine solution (0.01% v/v) for 2 min, rinsed in tap water for 1 min and dried. Samples were divided in 3 groups of replicates consisting of 13 fennel heads each. The 3 groups were processed one after another in order to avoid the occurrence of browning due to cutting operations. Each of the 13 fennel heads was cut into 8 slices of approximately 1 cm thickness by cutting with a sharp knife perpendicularly to the longitudinal axis, obtaining about 104 slices which were then randomly divided into 13 batches of 8 slices each. One batch was used for initial determinations. The remaining 12 batches were separated in 6 sub-batches (1 for each treatment) containing 2 batches each (one for each sampling day). Fennel slices were immersed for 2 min in one of the following solutions:

- 0.5 % (v/v) ethanol (ET);
- 1% (w/v) L-ascorbic acid (ASC);

- 0.5% (w/v) L-cysteine hydrochloride monohydrate adjusted to pH 7.0 with NaOH 1N (CYS);

- 1% (w/v) citric acid (CIT);

- 0.5% (w/v) 4-hexylresorcinol (HR);

- water as control (CTRL).

After the treatment slices were then dried with 2 layers of cheesecloth. Each sample of 8 fennel slices was placed in macro-perforated polyethylene clam-packs (119 x 189 x 90 mm; capacity 500 g; CL1/90 Carton Pack®), and stored at 5 °C. Initially, and after 2 and 6 days of storage samples were evaluated for the following quality parameters:

- sensorial attributes (appearance score, stem and sheath browning score, aroma, crunchiness, dehydration, overall quality);

- stem and sheath color;

- pH;

- total soluble solid (TSS);

- total phenols content;

- antioxidant activity;

- vitamin C (total, L-ascorbic and L-dehydroascorbic acid);

- microbiological quality (mesophilic bacteria, yeasts and moulds).

2.5.4 Results and discussion

The effect of treatment, time of storage (2 and 6 days) and their interactions on quality characteristics of fresh-cut fennels are shown in Table 2.5.4.1. Treatments

and storage time had a significant effect on almost all attributes, whereas Treatment x Time interaction was found statistically relevant only on few parameters (L* and a* on fennel stems, L* and hue angle on the sheathes, L-dehydroascorbic acid, total vitamin C and mesophilic bacteria).

All samples had appearance mean scores above the limit of marketability (3 or higher); the use of antibrowning agents in the water solution did not significantly improve the appearance of fresh-cut fennel, since no significant enhancement in the visual score of treated samples compared to CTRL was observed, rather the use of HR significantly worsened the appearance. Among antibrowning treatments, fennel slices dipped in ET had a significant higher score (4.1) compared to CIT and HR samples, whose mean scores were 3.2 and 3.1 respectively, while appearance score of fresh-cut fennels treated with ASC and CYS had intermediate values (Table 2.5.4.1).

Table 2.5.4.1 Effect of treatments, storage time and their interaction on quality parameters of fresh-cut fennel during storage at 5 °C. Data are mean values of 6 samples (3 replicates x 2 storage time).

Parameters	ET	ASC	CYS	CIT	HR	CTRL	Treatment	Time	Treatment X Time
Sensorial attributes									
Appearance score	4.1 a	3.6 abc	3.5 abc	3.2 bc	3.1 c	3.8 ab	***	****	ns
Stem browning	1.7 c	2.6 b	3.1 ab	3.6 a	3.1 ab	2.4 bc	****	****	ns
Sheath browning	1.1 b	1.6 ab	1.4 b	2.1 a	2.1 a	1.4 b	****	ns	ns
Aroma	3.0	2.7	2.6	2.6	2.4	2.7	ns	****	ns
Crunchiness	3.7	3.6	3.6	3.5	3.5	3.4	ns	*	ns
Dehydration	1.7	2.1	2.1	2.4	2.1	2.0	ns	****	ns
Overall quality	4.0 a	3.5 ab	3.4 ab	3.1 b	3.0 b	3.6 ab	*	****	ns
Physical attributes									
Stem color									
L*	90.4 a	87.1 b	85.4 bc	87.2 b	84.6 c	85.6 bc	****	****	****
a*	-0.2 b	0.5 a	0.4 ab	0.2 ab	0.3 ab	0.2 ab	*	****	*
b*	13.6 b	14.5 ab	15.4 ab	16.6 a	15.3 ab	14.1 b	**	****	ns
Chroma	13.6 b	14.5 ab	15.4 ab	16.6 a	15.3 ab	14.1 b	**	****	ns
Hue angle	91.4 a	88.5 b	89.0 b	89.8 ab	89.3 ab	89.6 ab	*	****	ns
Sheath color									
L*	89.1 a	88.4 a	85.0 b	86.9 ab	84.0 b	85.3 b	****	*	****
a*	-3.7 b	-2.7 a	-2.9 ab	-2.4 a	-2.9 ab	-3.1 ab	**	ns	ns
b*	13.0	12.4	13.4	13.2	13.6	13.2	ns	****	ns
Chroma	13.5	12.7	13.7	13.4	13.9	13.5	ns	****	ns
Hue angle	105.8 a	102.2 b	102.1 b	100.9 b	102.1 b	103.1 b	****	****	**
Chemical attributes									
Total soluble solid (°Brix)	5.4	5.0	5.3	5.0	5.2	5.2	ns	ns	ns
pH	6.4 a	6.2 b	6.3 ab	6.2 b	6.3 ab	6.2 ab	*	ns	ns
Total phenol content (mg GAE 100 g^{-1}fw)	18.3 c	28.1 a	24.4 ab	20.6 bc	21.1 bc	20.8 bc	***	*	ns
Antioxidant activity (mg TEAC 100 g^{-1}fw)	13.6 c	26.2 a	18.6 b	19.0 b	14.7 bc	15.0 bc	****	****	ns
Ascorbic acid (mg 100 g^{-1}fw)	6.0 b	16.3 a	6.1 b	5.5 b	5.4 b	5.4 b	****	***	ns
L-dehydroascorbic acid (mg 100 g^{-1}fw)	0.6 bc	2.6 a	0.7 b	0.8 b	0.3 c	0.6 bc	****	****	****
Vitamin C (mg 100 g^{-1}fw)	6.6 b	18.9 a	6.9 b	6.4 b	5.8 b	6.1 b	****	****	**
Microbiological quality									
Mesophilic bacteria (log CFU g^{-1})	4.2 bc	4.2 bc	4.4 bc	4.1 c	4.9 a	4.5 ab	***	****	***
Yeasts and moulds (log CFU g^{-1})	3.5	3.6	3.6	3.7	3.9	4.8	ns	****	ns

Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). Different letters indicate statistical differences among treatments, according to the Tukey's test ($p \leq 0.05$).

Antibrowning treatments significantly affected the browning score, for both stem and sheath cut-surfaces (Table 2.5.4.1). Mean score values for the stem browning

related to all sampling dates was significantly highest in CIT samples and lowest in ET samples. In the sheath portion highest browning score was observed for CIT and HR treatments while ET, CYS and CTRL samples had the lowest browning. Changes in browning scores for stem and sheath cut-surfaces during storage are described in Figure 2.5.4.1. On fennel stems the browning occurred regardless of the treatments and it significantly increased over time. After 2 days at 5 °C the stems in ET treatment had browning score of 1.1 ± 0.2, significantly lower compared to the scores of CIT (2.4 ± 0.5), HR (2.7 ± 0.6) and CYS (2.1 ± 0.2) samples. The same statistical differences among treatments were observed at the end of the storage, when fennel slices dipped in ET had a significant lower stem score (2.3 ± 0.7) than CIT (4.4 ± 0.7), HR (3.8 ± 0.7) and CYS (3.7 ± 0.8) samples, while ASC and CTRL samples has intermediate score values (3.3 ± 0.8 and 3.1 ± 0.9 respectively). In the sheath cut-surfaces browning was less evident and statistical differences among treatments were observed only after 6 day of storage; also in this case ET proved to be the most effective treatment (score 1.1 ± 0.2) although, similarly to ET, fennel slices dipped in ASC, CYS and water (CTRL) samples had score values below 2. Significantly higher level of browning in the sheath were scored in fresh-cut fennel dipped in CIT and HR solutions, compared to ET samples (Figure 2.5.4.1).

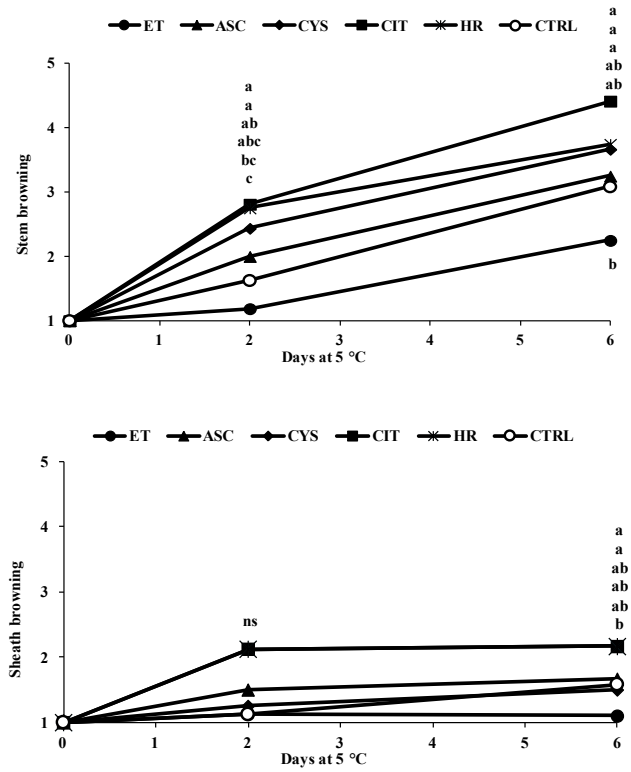

Figure 2.5.4.1 Effect of anti-browning treatments on sensorial evaluation of stem and sheath browning of fresh-cut fennel during storage at 5 °C. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Browning scale: 1= absence of browning, 3= slight browning, 5= completely brown.

Differences in browning appearance among treatments can be further clarified by considering results of instrumental color measurements which partially reflects the sensorial scores. Mean L* values for stem cut-surface related to all sampling dates were highest in ET samples while significantly lowest values were observed for HR

samples (Table 2.5.4.1). Regardless of the treatments, a general decrease in lightness occurred during storage, and significant differences were observed after 2 days at 5 °C where L* values in ASC, CIT and ET were significantly higher compared to CTRL, CYS and HR. At the end of the storage L* value in ET treatment was still significantly higher than ASC and CIT, while other treatments showed intermediate values (Figure 2.5.4.2). As shown in Table 2.5.4.1, ASC samples showed the highest mean a* value, and ET samples the lowest. Regardless of the treatments, a* values of the stem cut-surface moved from negative to positive values, indicating the switch from green to red components in the color (Figure 2.5.4.2). Significant differences in a* values were observed only after 6 days of storage where ASC samples had significant higher values compared to ET, while other treatments showed an intermediate behavior. Mean values of b* and chroma of the stem cut-surfaces was significantly lowest in ET and CTRL while fennel treated with CIT showed the highest b* and chroma values. ET samples showed highest hue angle values for stem cut-surface related to all sampling dates, while ASC and CYS samples (Table 2.5.4.1) showed the lowest values. Significant differences were observed after 6 days of storage where ET had significant higher values compared to ASC samples (Figure 2.5.4.2). These color results confirm that ET samples had the lower level of browning on fennel stem cut-surface but, differently from sensorial analysis, in which the higher browning score was in CIT samples, color data showed that the highest stem browning after 6 day of storage (lower L* and hue angle, and higher a*) was observed for ASC treatment.

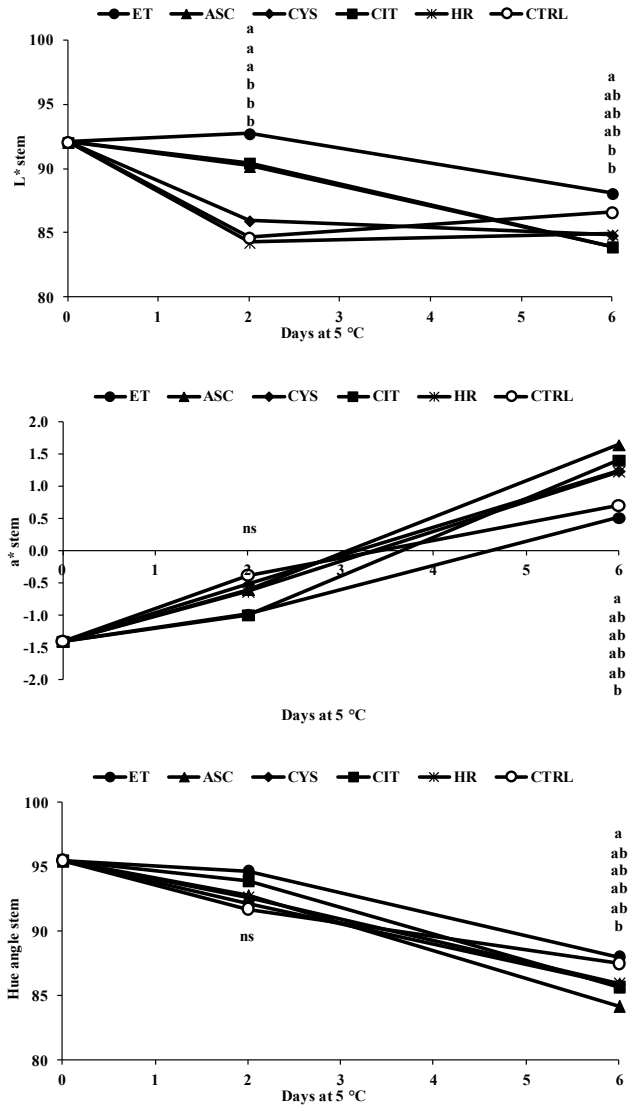

Figure 2.5.4.2 Effect of anti-browning treatments on L*, a* and hue angle values on stem of fresh-cut fennel during storage at 5 °C. Treatments: ET = ethanol, ASC = ascorbic acid, CYS = cysteine, CIT = citric acid, HR = 4-hydroxy resorcinol, CTRL = water. Values are mean of three replicates for each treatment. Within the

same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

L* values of the fresh-cut sheathes were significantly affected by treatments; mean L* values was the highest for ET and ASC samples, while the lowest L* values were observed in CYS, HR and CTRL samples (Table 2.5.4.1). Changes in L* values of the sheath cut-surface during storage are reported in Figure 2.5.4.3. After 2 day L* values for ET, AC and CIT were statistically higher compared to CTRL, CYS and HR; however at the end of the storage L* values did not show significant differences among treatments and mean values were slightly lower compared to the lightness of fresh samples (Figure 2.5.4.3). Mean a* values for sheath cut-surface related to all sampling dates was highest for ASC and CIT samples, while significant lowest values were observed for ET samples. An increase during storage was observed for all treatments, although remaining always in the negative side of the axis (Figure 2.5.4.3); no significant differences could be observed after 2 days while at the end of storage values for CIT were significantly higher (i.e. less negative) than for ET treatment. All other treatments showed an intermediate behavior. While b* value and chroma did not show significant differences among antibrowning treatments, hue angle resulted significantly highest (more green) in sheath cut-surface of ET samples, although for all treatments its mean values were higher than 90° (Table 2.5.4.1). Hue angle decreased throughout storage for all treatments but not for samples dipped in ET where it was more or less constant with values significantly higher than in all other treatments at day 2 (with exception of

samples dipped in CIT), but not at day 6, where the latter was the only treatment which showed a significantly lower value. These data indicate that discoloration in fennel sheath cut-surfaces takes place in all treatments (although at a much lower rate, compared to stem cut-surfaces browning) but in samples dipped in 0.5% ethanol, where all instrumental color attributes remained almost stable during storage (Figure 2.5.4.3). This confirms browning scores for CIT treatment but not for HR samples, which resulted not significantly different from the other treatments in terms of color parameters.

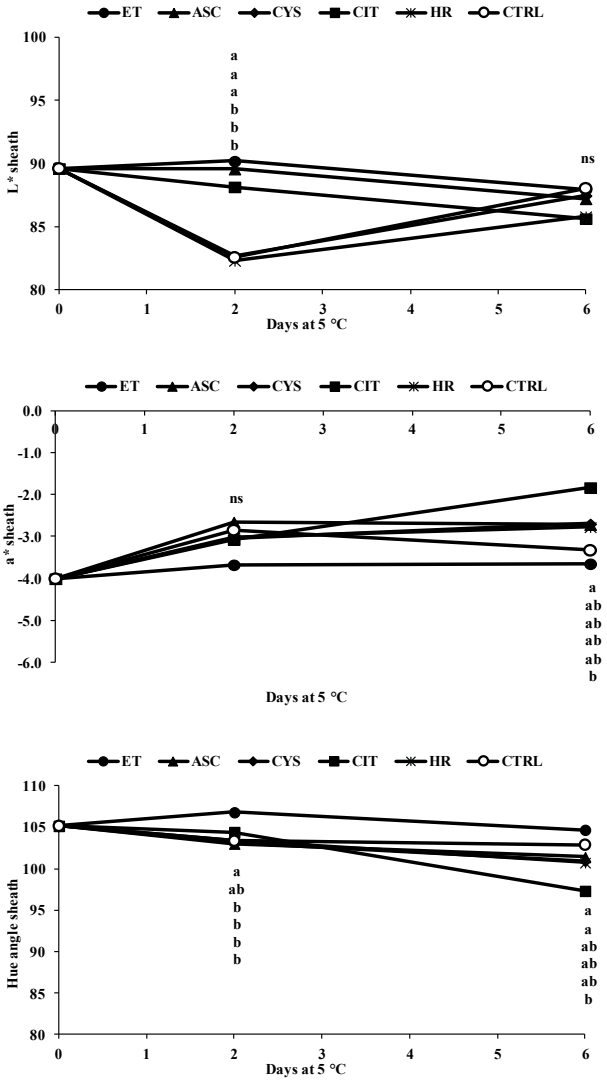

Figure 2.5.4.3 Effect of anti-browning treatments on L*, a* and hue angle values on sheath of fresh-cut fennel during storage at 5 °C. Treatments: ET = ethanol, ASC = ascorbic acid, CYS = cysteine, CIT = citric acid, HR = 4-hydroxy resorcinol, CTRL = water. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Enzymatic browning is one of the most limiting factors on the shelf-life of fresh-cut products and results from the action of a group of enzymes called polyphenol oxidases (PPO) that use phenolic compounds as substrate, leading to browning (Garcia and Barret, 2002). Several chemical compounds are reported to have inhibitory effects on enzymatic browning of various commodities. For instance, Roura et al. (2003) reported that dipping with ascorbic acid or citric acid did not improve the shelf-life of Romaine lettuce leaves, while their combination had a beneficial effect on the overall visual quality in terms of color, texture and brightness. Also Chiesa et al. (2001) observed an efficient browning inhibition of butter-head and iceberg lettuce stem by treatment with organic acid solution (1,5% citric acid + 1.5% ascorbic acid). The use of thiol-containing compounds, such as cysteine was found to retard browning of minimally processed fruits and vegetables such as pears (Sapers and Miller, 1998), banana (Moline et al., 1999), lettuce (Pace et al., 2014) and artichokes (Amodio et al., 2011). 4-hexylresorcinol was demonstrated to be effective in reducing browning of fresh-cut apples (Monsalve-González et al., 1993; Luo and Barbosa-Cánovas, 1995) and pears (Dong et al., 2000). Ethanol was also used as antibrowning agent by Wang et al. (2014) who applied a post-cut ethanol dipping at different concentrations on fresh-cut sunchoke tubers (Helianthus tuberosus L.) and after 15 days at 5 °C observed that all ethanol treatments significantly delayed browning compared to control in water. In addition 5, 8 and 10% ethanol dipped tuber slices retained the original color, and visual quality evaluation was confirmed by the differences in a* and hue angle values

compared to control. Regarding the application of antibrowning agents to reduce the browning of fennel cut-surface, few works are available. Albenzio et al. (1998) investigated the effectiveness of citric acid solution at different concentrations applied for 15 or 30 min, concluding that dipping fresh-cut fennels for 15 min in 0.1% citric acid is useful to delay the occurrence of browning of 5 day at 4 °C. Artés et al. (2002b) reported that treatments with 1% ascorbic acid and 5% citric acid did not control browning of butt-end cut-surface of whole fennels. Rinaldi et al. (2007) evaluated the effect of anti-browning dips on color evolution of cut-surfaces of fennel, finding that all the applied dipping solutions (citric acid, ascorbic acid, sodium chloride, cysteine, and ethanol, used alone or in various combinations) did not result in substantial improvement of color evolution in cut-surfaces compared to water-dip control; also in that case, some of them produced a more severe browning than control. In addition the same authors reported that dipping for 1 minute in 0.5% ethanol solution slightly delayed browning, while dipping for 1 minute in 1% citric acid + 0.5% cysteine solution, adjusted at pH 7, did not statistically differ from control.

The use of different antibrowning solutions had no effects on aroma, crunchiness and dehydration, but all of these attributes significantly changed during storage. Starting from score of 4.8 ± 0.2 in fresh samples, aroma decreased to an average value of 3.4 ± 1.2 after 2 days and further decreased at the end of the storage (average score 1.9 ± 1.1). The crunchiness of fresh samples was averagely scored 4.9 ± 0.1 and a slight loss of crunchiness occurred over time until a mean value of 3.3 ± 1.0 after 6 days of storage. As expected a slight dehydration occurred in all

samples due to the exposure of fennel slices to air during storage, and after 6 days the dehydration mean score was below 3. The overall quality of fresh-cut fennels was evaluated taking into account all the sensorial parameters analyzed and reflects similar statistical differences among treatments as the appearance scores (Table 2.5.4.1), although with slightly lower values as it was influenced by the loss of aroma and crunchiness occurred in all samples. Thus, the use of different antibrowning solution did not affect the overall quality of fresh-cut fennels compared to CTRL, and significant differences were observed in ET compared to CIT and HR samples (Table 2.5.4.1).

No changes in TSS were observed during storage and in relation to antibrowning treatments, while the pH was significantly higher in ET compared to ASC and CIT samples, probably because of the different pH of the dipping solutions.

Total phenolics and antioxidant activity resulted also affected by treatment. Initial total phenols content was 18.20 ± 1.94 mg GAE 100 g-1 fw; during cold storage the amount slightly increased and a significant effect of treatments was also observed (Table 2.5.4.1). ASC samples showed a mean content of 28.1 ± 3.6 mg GAE 100 g-1 fw, not different from CYS but significantly higher compared to all other treatments including ET which showed the lowest content of total phenols and which remained stable over time, having a mean value of 18.3 ± 3.2 mg GAE 100 g-1 fw. Taking into account the mode of action of the antibrowning tested in the present experiment, to understand the effect of these agents on changes in phenolic compounds it should be clarified the mechanisms involved in the phenolic metabolism that causes a variation of these compounds. It is well documented that

wounding due to cutting operations can stimulate phenolic metabolism in fresh-cut tissue (Saltveit, 2000; Klaiber et al. 2005). Wounding in fact induces the activity of PAL with consequent synthesis and accumulation of phenolic compounds; on the other hand these compounds can be oxidized by oxidative enzymes to o-quinones which ultimately polymerized to produce the browning appearance (Saltveit, 1997a; Tomás-Barberán et al., 1997; Degl'Innocenti et al., 2005). The Folin-Ciocalteu method measures phenolics capable of reducing the Folin-Ciocalteu reagent (Sánchez-Rangel et al., 2013) thus does not include those that are already oxidized by oxidative enzymes in the plant tissue.

The mechanisms of action of the anti-browning agents used in the present experiment are well documented: most of them act directly as inhibitors of PPO, others by reacting with the products of the PPO reaction, before these can lead to the formation of dark pigments (Garcia and Barret, 2002). Ascorbic acid prevents enzymatic browning by reducing the quinone products to their original polyphenol compounds (Walker, 1977). In the case of L-cysteine, the inhibition of the browning caused by PPO is carried out through a competitive mechanism by trapping o-quinones through the formation of cysteinyl adducts (Richard-Forget et al., 1992). In addition, similarly to ascorbic acid, Cilliers and Singleton (1990) described the ability of cysteine to reduce o-quinones to their polyphenols precursors. Citric acid has a double effect in inhibiting PPO: it works through a non-competitive mechanism, by chelating copper at the enzyme active site; in addition citric acid is an acidulant and serves the same purpose by lowering the pH below that necessary for the optimal enzyme activity (Ibrahim et al., 2004;

Altunkaya et al., 2008; Ali et al., 2015). The pH lowering can also be induced by ascorbic acid, although the effect is slightly less than citric acid (Garcia and Barret, 2002). 4-hexylresorcinol is a competitive inhibitors of PPO: it interacts with PPO to render an inactive complex incapable of catalyzing the browning reaction (Whitaket et al., 1995; Lambrecht, 1995). All of these anti-browning reagents act, in different way, on the enzyme PPO, possibly interfering with the oxidation of phenolic compounds. Differently, ethanol seems to act on the synthesis mechanism of phenolics: Yan et al. (2015) has recently reported that ethanol treatment is able to inhibit phenolic metabolism by repression of expression (mRNA) and activity of phenylalanine ammonia lyase (PAL), the key enzyme of phenolic biosynthesis. Thus, the increase of PAL activity may be considered the limiting factor for browning of cut fennels, as already demonstrated for fresh-cut lettuce (Campos-Vargas and Saltveit, 2002; Murata et al., 2004) and the effectiveness of ethanol treatment in delayed browning could be due to a lower synthesis of available substrates for oxidative reaction catalyzed by PPO. As shown for fresh-cut lettuce, in which the initial phenol content is very low, browning is the result of an active inductive process, requiring de novo synthesis of PAL and the consequent accumulation of phenolic compounds, rather than a passive oxidation of pre-existing phenols (Saltveit, 2000). Than the content changes of phenolic over time substantially depends on the balance of de novo synthesis and degradation, as previous reported by Vicente et al. (2011). Despite the other antibrowning treatments used in the present experiment have proved useful in delaying browning of several fruits and vegetables, they effectiveness depend on the concentration

used and on the commodity. In the present study none of the applied antibrowning treatments, except ethanol, were effective in control browning of the cut-surface of fresh-cut fennels, and this could be due to the concentration, both to the physiological response of the fennel tissue.

As for phenols, assuming that wounding stimulates the activity of PAL, treatments that inhibit PAL activity could reduce the amount of phenolic compounds, while those that inhibit the reactions in which are involved oxidative enzymes (i.e. PPO) may allow the accumulation of phenols. Thus, in samples treated with ethanol, phenolic compounds should not increase after wounding since this compound inhibits PAL activity, as reported above. Indeed in the present study total phenolics in ET samples remained steady during storage. These observations support the hypothesis that level of browning was significantly lower in fresh-cut fennel treated with ethanol, because there was less substrate available for PPO compared to samples of other treatments where phenolics slightly increased since none of them was able to directly inhibit PAL. This means that difference in phenolic content in these samples was mainly related to the mode of action of the selected antibrowning agent used. In the case of fresh-cut fennel treated with ASC and CYS, the highest total phenolics content could be due to the ability of these compounds to reduce the PPO activity and then determining in this way an increase in the amount of phenolics. In addition ascorbic acid may have interfered with the assay of phenolics by augmenting the effect on the amount of Folin-Ciocalteu reagent reacting with phenolic compounds (Sánchez-Rangel et al., 2013), therefore in ASC samples phenolics may have been overestimated. Differently from ASC and CYS, citric acid

and 4-hexylresorcinol do not act on the product of PPO therefore in these samples total phenolics content was very similar to CTRL. Results of the present study are partially in agreement with what shown by Altunkaya et al. (2009) on fresh-cut lettuce. These showed that ascorbic acid (0.5%) had a great capability for the prevention of the degradation of phenolics and the loss of these compounds was significantly lower compared to treatment with citric acid at the same concentration, water and L-cysteine 0.05%. In accordance with Altunkaya et al. (2009), in the present study ASC samples showed higher phenolic content compared to CIT and water samples (CTRL) but, differently from Altunkaya et al. (2009), no significant differences were observed comparing ASC and CYS. These differences were probably due to different antibrowning concentrations applied: the concentration ratio ASC/CYS in the present experiment was 2 while in Altunkaya et al. (2009) study the ASC/CYS ratio was 10. Therefore it is possible that the effect of L-cysteine depends on its concentration.

As for browning inhibition, also for phenolics metabolism the effectiveness of antibrowning compounds widely depends on their concentrations. A recent study by Ali et al. (2015) in lettuce explained how different concentration of ascorbic acid, citric acid and L-cysteine had a different impact on PPO activity: ascorbic acid could reduce the formed quinone instantly to the original substrate at high concentration (>1.5 %) while at lower concentrations acted as competitive inhibitor of PPO; also cysteine, at higher concentrations (≥1.0%) reacted with the resulted quinone to give a colorless products, while at the low concentrations cysteine

worked as competitive inhibitor of PPO. In addition this study confirmed that citric acid acted only as PPO non-competitive inhibitor.

As expected, in fresh-cut fennels treated with ascorbic acid, the vitamin C content resulted the highest, probably due to residues of ascorbic acid used during dipping operations. In particular after 3 days of storage ASC samples had significantly higher amount of AA and DHAA compared to others treatments and, as a consequences, same differences were observed in total vitamin C. At the end of the storage the AA content in ASC samples decreased although values were still significantly higher compared to CYS and CTRL, while the amount of DHAA was similar to other samples (Figure 2.5.4.4). These results are partially in agreement with the work of Gorny et al. (2002) on fresh-cut pears treated with ascorbic acid (2%). In that study the authors observed that, immediately after treatment, ascorbic acid levels were significantly higher in pear slices treated with ascorbic acid compared with control; however, after 3 days at 0 °C ascorbic acid residues on treated samples dropped to endogenous control levels. The authors explained this phenomenon since ascorbic acid was most likely converted to dehydroascorbic acid and then further degraded to 2,3 diketo-gluconic acid. This theory could explain why in the present experiment a decrease in AA after 6 days was not followed by an increase in DHAA.

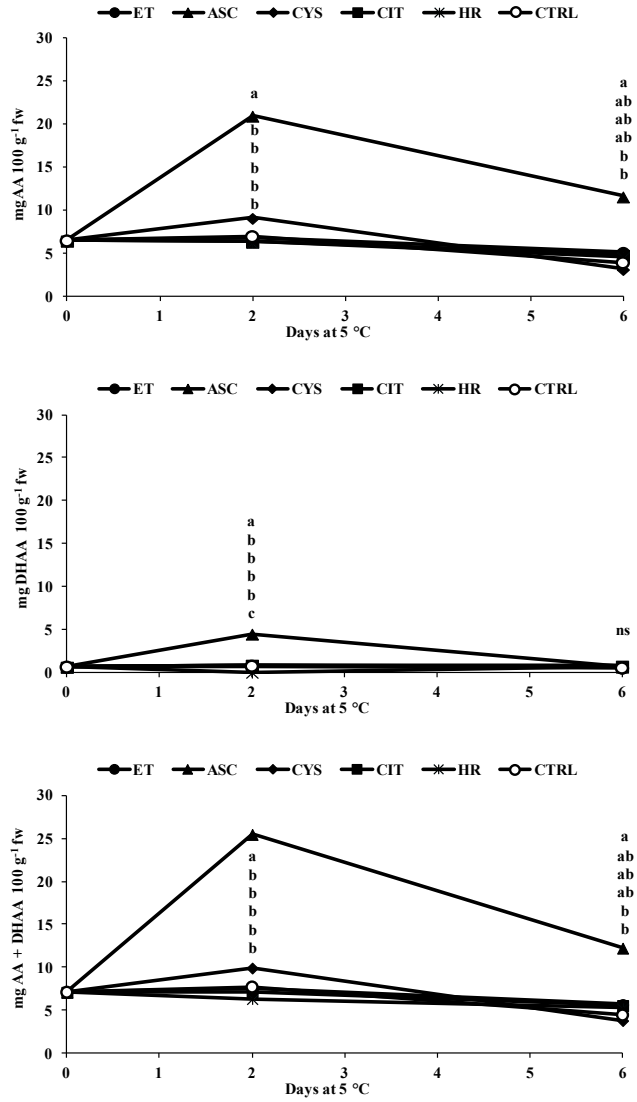

Figure 2.5.4.4 Effect of anti-browning treatments on vitamin C (AA, DHAA and AA+DHAA) content of fresh-cut fennel during storage at 5 °C. Treatments: ET = ethanol, ASC = ascorbic acid, CYS = cysteine, CIT = citric acid, HR = 4-hydroxy resorcinol, CTRL = water. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Treatment with ascorbic acid most probably influenced also the assay of antioxidant activity; in fact samples treated with ASC show the highest antioxidant capacity value (Table 2.5.4.1), because this organic acid act as an antioxidant in oxidative reactions in fresh-cut fruits and vegetables (Barth et al. 1993). However phenolic compounds have been established as the main contributors to the antioxidant activity of fruits and vegetable, and in general there is a high correlation between phenolics and antioxidant activity (Rice-Evans et al., 1996; Jacobo-Velázquez and Cisneros-Zevallos, 2009), therefore it is expected to have a low antioxidant activity in samples with a low content in phenolic compounds. Accordingly, the lower antioxidant activity was measured in fresh-cut fennels treated with ethanol where no increase in phenolics was observed during storage. Others treatments (CYS, CIT, HR and CTRL) had similar antioxidant activity values and, as for phenolics, no significant differences were observed among them (Table 2.5.4.1). An high correlation (R2 = 0.97) between total phenolic and DPPH scavenging activity was found in two sweet fennel cultivars, Dulce and Zeta fino, as reported by Salama et al. (2013). The same authors also observed an high R2 (0.97) comparing vitamin C and DPPH scavenging activity however in their experiment the amount of vitamin C measured was averagely 3 times higher compared to that in fennel used in the present experiment, therefore its contribute to the antioxidant activity assay was certainly greater.

Some of the anti-browning agents used in the present experiment are recognized to have antimicrobial properties (Yildiz, 1994; Beaulieu and Baldwin, 2002; Rico et

al., 2007; Herppich et al., 2014; Erginkaya et al., 2014; Yan et al., 2015). For instance organic acids, such as citric acid and ascorbic acid, exert an antimicrobial action due to pH reduction on the surface of cut products (Beaulieu and Baldwin, 2002; Beuchat, 2000); ethanol kills microorganisms by denaturating their proteins and dissolving their lipids (Mcdonnell and Denver, 1999). Therefore it is interesting to evaluate the effect of these compounds on the microbial growth in fresh-cut fennel during storage. As shown in Table 2.5.4.1, treatments did not affect the growth of yeasts and moulds; in fact these microorganisms increased during storage of about 2 log in all samples, without significant differences among treatments. However significant effects of treatment, time and of their interaction were observed on mesophilic bacteria (Figure 2.5.4.5). The initial mesophilic bacteria counts were 3.5 ± 0.5 log CFU g-1 and the loads increased in all samples during storage, with significant differences among treatments observed after 6 days at 5 °C only in fresh-cut fennel dipped in 4-hexylresorcinol solution, that had significant higher mesophilic counts (5.5 ± 0.003 CFU g-1) compared to samples treated with citric acid (4.4 ± 0.2 CFU g-1). The effectiveness of citric acid in delaying microbial population was previously reported although depending on the concentration used. For instance Ibrahim et al. (2009) observed that leaves of some selected vegetables decontaminated with 5% citric acid showed a considerable decrease in microbial count compared to water washing, while a treatment with 0.2% citric acid on iceberg lettuce leaves was not able to reduce microbial population compared to water (Kim et al., 2011). In the same experiment on lettuce, treatment with 50% ethanol spray reduced the microbial count of about 2 log after 6

days at 5 °C compared with control in water (Kim et al., 2011). A short term (30 sec) 50% ethanol dipping at 10 °C immediately reduced total bacterial and mould counts in white asparagus spears (Asparagus officinalis L.) and retarded their growth during storage (Herppich et al., 2014). Oh and Marshall (1993), investigating the antimicrobial effect of ethanol at different concentrations against Listeria monocytogenes, concluded that up to 1.25% ethanol did not inhibit growth; on the other hand growth was strongly inhibited in the presence of 5% ethanol. Therefore, as for citric acid, also the effectiveness of ethanol could depend on the applied concentrations. This hypothesis is supported also by Allende et al. (2009) who observed that antimicrobial activity of ascorbic acid in cut cilantro was significantly influenced by the applied concentration: the reduction in microbial counts increased with the increase in ascorbic acid concentration. On the base of microbial results of the present experiment, none of the antibrowning treatments applied during dipping of fresh-cut fennel was able to significantly delay the growth of mesophilic bacteria, yeast and moulds, compared to control in water, and it was probably due to low concentrations used.

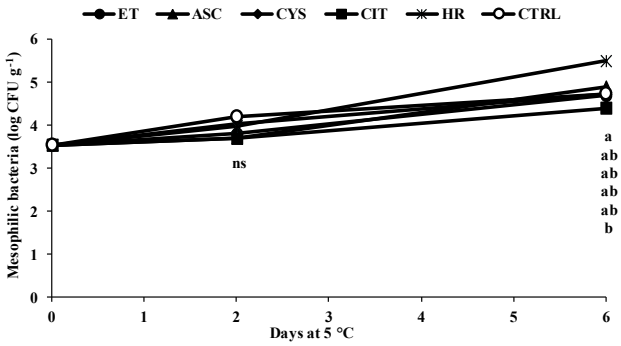

Figure 2.5.4.5 Effect of anti-browning treatments on mesophilic bacteria growth in fresh-cut fennel during storage at 5 °C. Treatments: ET = ethanol, ASC = ascorbic acid, CYS = cysteine, CIT = citric acid, HR = 4-hydroxy resorcinol, CTRL = water. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

2.5.5 Conclusions

Result of the present study showed that, among all applied antibrowning agents, dipping in 0.5% ethanol was effective in preserving visual quality of fresh-cut fennel stored in air for 6 days at 5 °C, significantly reducing the browning in both stem and sheath cut-surfaces. In addition ethanol is a GRAS product and it did not negatively influence other sensorial parameters analyzed in the present experiment, such as aroma. Based on these considerations, the dipping in 0.5% ethanol for 2 min could be a useful pretreatment for extending the shelf-life of fresh-cut fennel.

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2.6 EFFECTS OF CONTROLLED ATMOSPHERE ON QUALITY AND SHELF-LIFE OF FRESH-CUT FENNELS cv. APOLLO

2.6.1 Abstract

Objective of this study was to understand the effects of atmosphere modification on fresh-cut 'Apollo' fennel and to identify best suitable gas mixture to extend its shelf-life. Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Apollo*) were trimmed, sliced, dipped in EtOH 0.5% as antibrowning agents, and stored for 14 days at 5 °C in different controlled atmosphere (CA) conditions. Two different experiments were carried out. In the first experiment the applied CA conditions were the following: 2 kPa O_2 in nitrogen, 20 kPa CO_2 in air, 2 kPa O_2 + 20 kPa CO_2 in nitrogen, and Air (as a control). Sensorial (appearance score, stem and sheath cut surfaces browning score), physiological (respiration rate), physical (stem and sheath cut-surfaces color, weight loss), chemical (TSS, pH, phenolic, antioxidant activity, vitamin C, ethanol, acetaldehyde contents) and microbiological (mesophilic bacteria, yeasts and moulds counts) attributes were evaluated at time 0 and after 3, 9 and 14 days. In the second experiment fresh-cut fennels were stored at 5 °C in the following CA conditions: 5 kPa O_2 in nitrogen, 5 kPa CO_2 in air, 10 kPa CO_2 in air, 5 kPa O_2 + 20 kPa CO_2 in nitrogen. Color evaluation was performed at time 0 and after 3, 9 and 14 days. Results of the first experiment showed that an atmosphere of air enriched with 20 kPa CO_2 was effective to preserve the appearance of fresh-cut fennel stored at 5 °C for 14 days, delaying the occurrence of browning on the cut surface of fennel slices. When the oxygen level was decreased

to 2 kPa in the presence of 20 kPa CO_2, the effectiveness of CO_2 on controlling stem browning slightly decreased. Results from a wider range of atmosphere compositions tested in the second experiment to further clarify the effect of atmosphere modifications on total color variation of the cut surface substantially confirmed previous finding. When only O_2 concentration was lowered no control of browning was observed, with a similar loss of visual quality as detected in control samples stored in air. From a nutritional point of view no significant changes were observed in terms of antioxidant capacity, phenolic and ascorbic acid contents in relation to the applied CA conditions. On the other hand the microbiological quality was significant influenced by the presence of CO_2 as the growth of mesophilic bacteria was delayed; the lowering of oxygen seemed to be not effective on the inhibition of mesophilic population while it affected the count of yeasts, retarding their growth. Taking into account the overall quality parameters of fresh-cut fennel over time, the model obtained using multivariate analysis confirmed that samples stored in air enriched with 20 kPa CO_2 showed the slowest degradation kinetic. However, it should be considered that the production of fermentative metabolites could occur in this storage condition. In addition, an atmosphere of 20 kPa CO_2 in air is not feasible to be obtained in modified atmosphere packaging (MAP),since due to the film permeability, the CO_2 accumulation cannot be unrelated to oxygen consumption.

2.6.2 Objective

The aim of this study was to understand the effects of atmosphere modification on fresh-cut 'Apollo' fennel and to identify best suitable gas mixture to extend its shelf-life. In a first experiment the following atmosphere compositions were tested: 2 kPa O_2 in nitrogen, 20 kPa CO_2 in air, and their combination 2 kPa O_2 + 20 kPa CO_2 in nitrogen. In a second experiment, using the same fennel cultivar, the following conditions were applied for to better understanding the effect on color changes during storage: 5 kPa O_2 in nitrogen; 5 kPa CO_2 in air; 10 kPa CO_2 in air; 5 kPa O_2 + 20 kPa CO_2 in nitrogen).

2.6.3 Experimental setup

Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Apollo*) were harvested on April 2014 in Puglia (Italy) and transported in cold condition to the Postharvest laboratory of the University of Foggia where they were stored at 0° C until processing. After trimming operations, fennels were washed in chlorine solution (0.01% v/v) for 2 min, rinsed in tap water for 1 min and dried. Each fennel head was then cut into slices of approximately 1 cm of thickness by cutting perpendicular to the longitudinal axis with a sharp knife, and the slices were kept in water up to the formation of the experimental lots. Fennel slices were immersed for 2 min in 0.5% ethanol solution, which proved the most effective as anti-browning solution in previous experiments, and dried with 2 layers of cheesecloth. Three replicates of 24 slices were kept for the initial determinations, while the remaining slices were divided in 36 groups of 24 slices and placed in macro-perforated

polyethylene clam-packs (119 x 189 x 110 mm; capacity 750 g; CL1/110 Carton Pack®). Groups of 3 macro-perforated polyethylene clam-packs (one for each storage duration) were placed in 15 L jars, for a total of 12 jars (3 replicates for each of 4 storage conditions). All jars were then placed in a 5 °C room and a gas system was used to inject a continuous and humidified flow (0.2 L min^{-1} and 95% RH) of air (as control), 2 kPa O_2 in nitrogen, 20 kPa CO_2 in air, and 2 kPa O_2 + 20 kPa CO_2 in nitrogen. Sampling was performed after 3, 9 and 14 days and the following attributes were analyzed:

- respiration rate;
- sensorial analysis;
- stem and sheath color;
- weight loss;
- total soluble solid (TSS);
- pH;
- titratable acidity (TA);
- total phenols content;
- antioxidant activity;
- vitamin C (total, L-ascorbic and L-dehydroascorbic acid);
- acetaldehyde and ethanol content;
- microbiological analysis.

For the second experiment the same fennel cultivar (*Apollo*) harvested in the same field but 2 weeks later was used. Fennel heads were processed and stored as reported above, but flushing different gas compositions. In particular 12 jars (3

replicates for each of 4 storage conditions), each containing 3 macro-perforated polyethylene clam-packs with fresh-cut fennel samples (1 clam-pack for each sampling time) were placed in a 5 °C room and a continuous and humidified flow (0.2 L min^{-1} and 95% RH) of 5 kPa O_2 in nitrogen, 5 and 10 kPa CO_2 in air, and 5 kPa O_2 + 20 kPa CO_2 in nitrogen were injected. Total color variation (ΔE) on stem and sheath part of fennel slices were evaluated after 3, 9 and 14 days of storage.

In addition to the multifactor ANOVA test used for statistical analysis, the interpretation of the experimental data of the present study was improved using a multivariate approach (multivariate accelerated shelf-life testing, MALST) in order to estimate the differences in the overall degradation of fresh-cut fennel quality parameters.

2.6.4 Results and discussion

Table 2.6.4.1 shows the effect of treatments (gas composition), time of storage and their interactions on quality attributes of fresh-cut fennels cv. *Apollo*. Gas treatments influenced visual quality, browning of the stem and sheath and the overall quality of fennel slices as well as respiration rate and all the color parameters analyzed in both stem and sheath. Also gas compositions significantly affected some chemical attributes such as total soluble solid, titratable acidity, the contents of ethanol and acetaldehyde and the microbial quality. Time of storage affected most of the attributes, except for a* value and hue angle in both stem and sheath, and the contents of total soluble solid, titratable acidity and total phenolics content. The interaction between time of storage and treatment significantly

affected the overall quality and the values of a* and hue angle in the stem. Also a significant effect of interaction time x treatment was found on the contents of DHAA, ethanol, acetaldehyde as well as in the amount of total mesophilic bacteria, yeasts and moulds.

Table 2.6.4.1. Effect of treatments (gas composition), storage time and their interaction on quality parameters of fresh-cut fennel during storage at 5 °C. Data are mean values of 9 samples (3 replicates x 3 storage time).

Parameters	AIR	2 kPa O_2	2 kPa O_2 + 20 kPa CO_2	20 kPa CO_2	Treatment	Time	Treatment X Time
Sensorial attributes							
Visual appearance	2.4 b	2.7 b	3.4 a	3.3 a	****	***	ns
Stem browning	3.5 a	3.1 a	2.2 b	2.0 b	****	*	ns
Sheath browning	2.0 a	1.8 ab	1.7 b	1.7 b	*	****	ns
Aroma	2.2	2.1	2.3	2.3	ns	****	ns
Crunchiness	3.8	3.8	3.8	3.8	ns	*	ns
Dehydration	2.5	2.3	2.2	2.4	ns	****	ns
Flavour	2.9	3.0	2.8	2.8	ns	**	ns
Sweetness	2.4	2.5	2.4	2.4	ns	****	ns
Overall quality	2.5 b	2.8 a	3.0 a	2.9 a	**	****	**
Physiological attributes							
♯ Respiration rate (mL CO_2 kg^{-1} h^{-1})	9.9 a	6.2 b	-	-	**	*	ns
Physical attributes							
Weight loss (%)	0.6	0.4	0.4	0.4	ns	****	ns
Stem color							
L*	84.7 b	85.3 b	91.6 a	91.7 a	****	****	ns
a*	1.7 a	1.1 b	0.3 c	-0.4 d	****	ns	*
b*	16.0 a	16.6 a	14.2 b	12.9 c	****	****	ns
Chroma	16.1 a	16.6 a	14.2 b	12.9 c	****	****	ns
Hue angle	83.9 d	86.3 c	88.8 b	91.8 a	****	ns	*
Sheath color							
L*	88.3 ab	87.7 b	89.9 a	89.4 ab	*	****	ns
a*	-3.2 a	-3.5 ab	-3.3 ab	-3.9 b	*	ns	ns
b*	13.9 a	12.9 ab	11.6 c	12.2 bc	****	**	ns
Chroma	14.2 a	13.3 ab	12.1 c	12.8 bc	***	**	ns
Hue angle	102.9 c	105.0 b	105.8 ab	107.7 a	****	ns	ns
Chemical attributes							
Total soluble solid (°Brix)	5.3 b	5.8 a	5.6 ab	5.4 b	***	ns	ns
pH	6.7	7.2	6.9	6.7	ns	***	ns
Titratable acidity (mEq NaOH 100 g^{-1} fw)	1.3 a	1.3 ab	1.1 bc	1.1 c	****	ns	ns
Total phenol content (mg GAE 100 g^{-1} fw)	21.3	23.0	20.2	19.8	ns	ns	ns
Antioxidant activity (mg TEAC 100 g^{-1} fw)	20.1	20.7	19.1	19.8	ns	****	ns
Ascorbic acid (mg 100 g^{-1} fw)	5.5	6.3	7.7	6.7	ns	**	ns
L-dehydroascorbic acid (mg 100 g^{-1} fw)	0.7	0.5	0.5	0.7	ns	**	****
Vitamin C (mg 100 g^{-1} fw)	6.1	6.9	8.3	7.4	ns	*	ns
Ethanol (ul L^{-1})	17.6 b	5.3 b	56.6 b	256.0 a	****	**	****
Acetaldehyde (ul L^{-1})	4.1 c	2.9 d	5.3 b	10.2 a	****	****	****
Microbiological quality							
Mesophylic bacteria (log CFU g^{-1})	7.9 a	7.1 b	6.2 c	7.1 b	****	****	**
Yeasts and Moulds (log CFU g^{-1})	7.0 a	6.5 b	6.0 c	5.8 c	****	****	****

Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). Different letters indicate statistical differences within treatments, according to the Tukey's test ($p \leq 0.05$).

♯ data of respiration rate are only related to samples stored in air and in 2 kPa O_2 in nitrogen.

At each sampling time, the first parameter measured was the rate of respiration. A slight effect ($p \leq 0.05$) of time on respiration rate was observed: starting from 14.6 ± 0.1 mL CO_2 kg^{-1} h^{-1} 24 h after cutting, respiration rate decreased during time reaching, after 14 days at 5 °C, mean values of 8.2 ± 2 mL CO_2 kg^{-1} h^{-1} and 4.9 ± 2 mL CO_2 kg^{-1} h^{-1}, in AIR and 2 kPa O_2 respectively. The higher CO_2 production at the beginning of the storage was probably correlated to the mechanical damage applied by cutting. It is reported that the wounding of plant cells and tissues, due to mechanical damage or cutting may cause an increase in the respiration rate (Fonseca et al., 2002). The increase in respiration in wounded plant tissues is thought to be a consequence of elevated ethylene, which stimulates respiration (Brecht, 1995). Regardless treatments, in our experiment a significant reduction in respiration rate was observed during storage. Similar trend in respiration rate was previously reported on sliced fennel cv. Clio stored at 5 °C (Escalona et al., 2005b), and on fresh-cut fennels cv. Orion stored at 0 °C (Escalona et al., 2005a; Escalona et al., 2006). The same authors measured respiration rate also on intact fennel, founding a significant effect of cutting operations on respiration rate: the effect of cutting resulted in a CO_2 production 1.5-fold higher than that in the whole fennel (Escalona et al., 2005a). A significant effect of treatment on respiration rate was also observed (Table 2.6.4.1). Respiration rates of fresh-cut fennel stored under low-oxygen conditions resulted lower than sample stored in AIR. Saltveit (2003) also reported that decrease in O_2 concentration as well as increases in CO_2 concentration lead to a decrease in the respiration rate of fruits and vegetables. Our

results are in agreement with data reported by Escalona et al. (2006) that observed a significant higher respiration activity in fennel slices cv. Orion stored at 5 °C for 13 days in air compared to slices stored in CA with 5 kPa O_2 + 15 kPa CO_2 or 5 kPa O_2 + 5 kPa CO_2 at 5 °C. Similar results were also reported by Artés et al. (2002) for whole fennel cv. Orion in which the respiration rate at 5 °C was reduced when O_2 levels decreased from 21 to 5%. In addition the effect of low oxygen on the reduction of respiration rate are reported on several fresh-cut products such as green onions (Hong et al., 2001), bell peppers (Conesa et al., 2007b), and pineapple (Marrero et al., 2006).

As expected there was a significant effect of storage time on weight loss, while no differences between treatments were found for this parameter (Table 2.6.4.1). In general the loss of weight was less than 1% as a high relative humidity (95%) was maintained in the containers throughout the experiment. In fact it is well known that the relative humidity control can help minimize the rate of water loss (Garcia et al., 2002).

All sensorial attributes were significantly affected by storage time while CA treatments only influenced visual attributes including appearance score, browning scores on stem and sheath, and the overall quality score (Table 2.6.4.1). In particular, while samples stored in high CO_2, in air or in combination with low O_2, showed appearance score values above the limit of marketability, samples stored in air and in low-O_2 atmosphere had significantly lower values, although in both cases they were still edible. Considering that only a very slight dehydration occurred during time, without differences between treatments, and no microbial spoilage was

detected, the main cause of quality loss and decreased marketability of sliced fennels was to be attributed to the change of color, possibly due to the occurrence of browning as showed by data related to browning scores both for sheath and stem cut surfaces. The presence of 20 kPa CO_2 in the gas mixture significantly reduced browning incidence, while low oxygen CA condition, without additional high-CO_2, (2 kPa O_2) did not show browning scores significantly different from samples stored in air.

Visual score data are in agreement with results reported by Escalona et al. (2006) on fresh-cut fennels cv. Orion where control samples stored in air had lower visual scores compared to samples stored under CA conditions (5 kPa O_2 + 15 kPa CO_2). Regardless to the effect of treatments, browning scores attributed to the sheath cut surface were always lower (ranging from 1 to 2.4) compared to those given to stem cut surface, which ranged from 2 to 3.9, meaning that browning occurred mostly on stem portions of fennel slices; this is probably the reason why browning of the butt-end cut is one of the most important factors affecting visual quality of whole fennel heads (Artés et al., 2002).

Results of browning scores as perceived by panelists were supported by color data. In fact, treatments with 20 kPa CO_2 confirmed its effectiveness in delaying browning of cut stem parts of fennel slices as shown by significant higher values of L* and of hue angle as well as lower a* and b* values compared to samples held in air and in CA with 2 kPa O_2. In addition color data also underline significant differences between treatments with 20 kPa CO_2 that were not appreciated by panelist: stems of fennel slices stored in 20 kPa CO_2 in air had lower a* value and

higher b*, chroma and hue angle values compared to samples held in 20 kPa CO_2 + 2 kPa O_2, while the lightness (L*) remained similar in both treatments. On the stem cut surface significant interactions between time and treatments were detected for a* and hue angle values for which changes over time were reported in Figure 2.6.4.1. After 3 days at 5 °C samples stored in air and in CA with 2 kPa O_2 had a statistically significant increase in a* and a decrease of hue angle compared to fresh-cut fennel stems in CA with 20 kPa CO_2 (in air or in low oxygen) in which a* values still remained negative. Afterwards only slight further changes in a* and hue angle values were observed in samples stored in air or in 2 kPa O_2 while in fennel slices held in 2 kPa O_2 +20 kPa CO_2, a* moves from negative to positive values and a gradual decrease in hue angle was also observed. At the end of storage samples held in CA with 2 kPa O_2, with or without 20 kPa CO_2, had similar a* and hue angle values, significantly different either from AIR and 20 kPa CO_2 treatments. In fact, after 14 days of storage at 5 °C, fresh-cut fennels stored in 20 kPa CO_2 in air showed a* and hue angle values similar to fresh samples, while AIR treatment showed the highest changes in these color parameters (Figure 2.6.4.1).

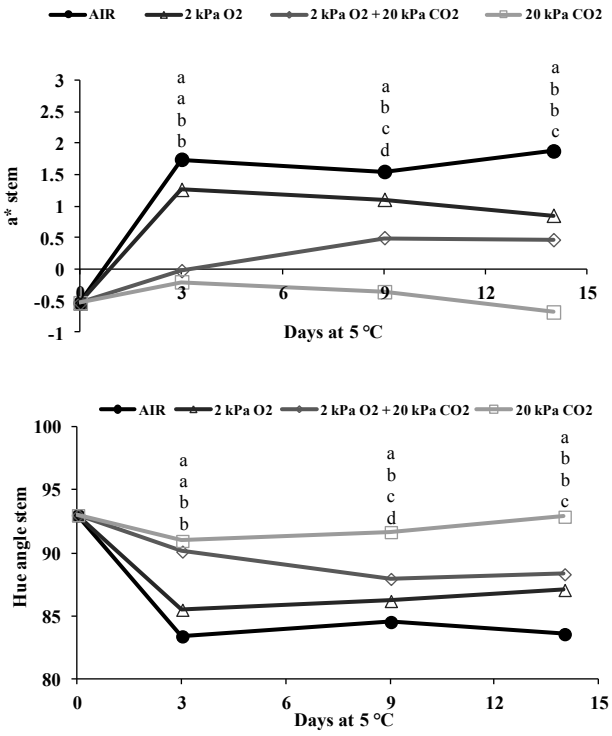

Figure 2.6.4.1 Changes in a* and hue angle values in stem part of fresh-cut fennel stored under different controlled atmospheres for 14 days at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

A similar effect of carbon dioxide on color parameters were also observed for the sheath cut-surface, even if statistical differences were less consistent. Hue angle showed lower decrease in fresh-cut fennel stored in 20 kPa CO_2 in air or + 2 kPa O_2 but only for the former differences from treatments in 2 kPa O_2 and AIR were statistically significant. As for stems also on the sheathes b* values were higher in AIR or 2 kPa O_2 CA compared to treatments stored in high CO_2 (Table 2.6.4.1).

Regardless treatments, browning occurred more intensively on stems than on sheathes, according to browning scores reported above. In fact a* values of the sheathes remain always negative, indicating no disappearance of green color, while on the stems they move from negative to positive values, except in fresh-cut fennel stems held in 20 kPa CO_2 in air.

From color data analysis it may be concluded that 20 kPa CO_2 in the gas mixture significantly delayed browning of the cut surface of fresh-cut fennel during storage, and the effect was more evident on the stems than on the sheathes. When the oxygen level was decreased to 2 kPa in the presence of 20 kPa CO_2, the effectiveness of CO_2 slightly decreased, especially in the case of the stem cut surfaces. The effect of CO_2 in inhibiting browning was previously reported on different crops (Murr et al., 1974; Buescher et al., 1977; Siriphanich et al., 1985; Dong et al., 2015) and is due to its ability in inhibiting or reducing the activity of PPO (polyphenol oxidase). PPO is one of the main enzyme involved in the browning reaction, since it catalyzes the oxidation of phenolic compound with subsequent formation of dark pigments (Tomás-Barberán et al., 2001). Therefore reducing or inhibiting the activity of PPO, CO_2 could reduce or avoid the occurrence of browning. Concerning the effect of low oxygen, in the present study the application of a CA with 2 kPa O_2 resulted slightly better than control in prevent browning, probably because a decrease in oxygen availability can affects the PPO activity. In fact it is well known that PPO requires oxygen in order to induce cut surface discoloration (Beaulieu et al., 2002), so reducing the oxygen levels in fresh-cut products atmosphere may reduce browning of the cut surface. When low oxygen

and high CO_2 were combined in the CA treatment (2 kPa O_2 + 20 kPa CO_2) the beneficial effect of gas composition in preventing browning of fresh-cut fennel was enhanced compared to that in 2 kPa O_2 atmosphere, but resulted similar compared to the treatment with 20 kPa CO_2 in air. To better understand the effect of different gas compositions on color changes of fennel cut-surfaces, color parameters of both CA experiments were compared. To minimize the color variability due to a different raw material, the comparison was made using the total color variation (ΔE) calculated from the initial L*, a* and b* values of samples from each experiment. The effect of atmosphere composition, time of storage and of their interactions on total color variation (ΔE) on stem and sheath cut-surface is reported in Table 2.6.4.2. The highest ΔE values for fennel stem cut-surfaces was observed in AIR and in samples held in low oxygen CA treatments, while lower color variations were found in fennel slices stored in air enriched with CO_2 (5, 10 and 20 kPa CO_2). The decrease in oxygen to 2 or 5 kPa O_2 in the presence of 20 kPa CO_2, slightly increased the total color variation in fennel stem cut-surfaces. These results clarify that only carbon dioxide was effective in delayed browning of the stems in fresh-cut fennels. The effect of gas composition on the total color variation in the fennel sheath cut surfaces was less evident, however CA conditions with 20 kPa CO_2 in air resulted in significantly less color changes compared to AIR and 5 kPa CO_2.

Table 2.6.4.2 Effect of treatments (gas composition), storage time and their interaction on total color variation (ΔE) on stem and sheath of fresh-cut fennel during storage at 5 °C. Data are mean values of 9 samples (3 replicates x 3 storage time).

Treatments	ΔE stem	ΔE sheath
Treatments		
2 kPa O_2	8.84 a	2.76 ab
5 kPa O_2	8.11 a	4.12 a
5 kPa CO_2	3.51 bc	3.27 ab
10 kPa CO_2	2.80 c	3.29 ab
20 kPa CO_2	3.42 bc	2.55 b
2 kPa O_2 + 20 kPa CO_2	4.67 b	3.53 ab
5 kPa O_2 + 20 kPa CO_2	5.24 b	3.85 ab
AIR	8.55 a	4.19 a
Treatment	****	**
Time	***	ns
Treatment x Time	***	****

Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). Different letters indicate statistical differences within treatments, according to the Tukey's test ($p \leq 0.05$).

Results of the present study are partially in agreement with data reported by Artés et al. (2002) that observed a significant inhibition of browning in the butt-end cut surface of whole fennels cv. Orion stored in CA with 5 kPa O_2 + 5 kPa CO_2 compared to 5 kPa O_2 after 11 days at 0 °C, however this effect was not evident at 5 °C. In addition, regardless CA treatments, no browning was observed in external leaves, both at 0 and 5 °C. Escalona et al. (2006) also observed a significant

reduction of browning of sliced fennels cv. Orion when the carbon dioxide concentration was increased from 5 to 15 kPa in the presence of 5 kPa O_2. These studies confirmed our findings on the effectiveness of CO_2 in delayed browning of fennels.

Beside changes in visual appearance, stems and sheathes browning score as well as dehydration, others sensorial attributes affects the overall quality of fresh-cut fennels under different CA storage. Very low changes were found during time for crunchiness score, probably thanks to the high relative humidity used during storage that avoided excessive dehydration of fennel slices (Table 2.6.4.1). Aroma, flavor, and sweetness scores decreased over time with no significant difference found among samples stored under different atmosphere treatments (Table 2.6.4.1). As reported in Table 2.6.4.1, the overall quality was influenced either by atmosphere composition and by storage time; in particular AIR samples had the lowest mean value (2.48) while no significant differences were observed among the other treatments. Considering that for these attributes a significant interaction time x treatment was found, the effect of atmosphere modifications at each sampling time is shown in Figure 2.6.4.2.

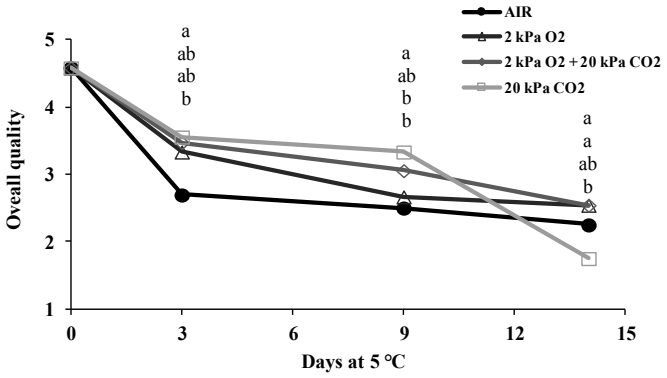

Figure 2.6.4.2 Overall quality of fresh-cut fennel stored under different controlled atmospheres for 14 days at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Scale: 5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

The overall quality decreased for all samples during storage. After 3 days, samples stored in 20 kPa CO_2 in air had significant higher score (3.6) compared to fresh-cut fennel in air (2.7). In these samples the overall quality scores slightly decreased until day 9 when the same statistical differences were maintained; in addition the overall quality of samples stored with 2 kPa O_2 decreased from a score of 3.3 after 3 days, to 2.7 at day 9 when it resulted significantly lower compared to 20 kPa CO_2 in air. At the end of storage (14 days) all samples has scores below 3; in particular samples stored with 20 kPa CO_2 in air showed a dramatic decrease in overall quality at last sampling time, reaching the lowest score of 1.8, probably due to a slight smell of fermentation observed by the panelists. These observations were partially confirmed by data analysis of ethanol that significantly increased at the

end of the storage. As reported in Figure 2.6.4.3 an increase in products of fermentative metabolism was detected in samples stored with high CO_2 in air. The contents of acetaldehyde and ethanol were significantly higher in fennels treated with 20 kPa CO_2 in air compared to all other samples, for each sampling day. These results suggests that 20 kPa CO_2 in fresh-cut fennel is able to induce the activation of fermentative metabolism. However when the oxygen level was decreased, even maintaining the same CO_2 concentrations (2 kPa O_2 + 20 kPa CO_2), fermentation did not occurred. Also fresh-cut fennels stored in CA with 2 kPa O_2 did not show any increase in ethanol and acetaldehyde. To explain this unexpected phenomena it is important to underline that fermentative metabolism includes two pathways. In one pathway, pyruvate is decarboxylated to form acetaldehyde, catalyzed by the enzyme PDC (pyruvate decarboxylase); then the enzyme ADH (alcohol dehydrogenase) reduced acetaldehyde to ethanol, using NADH. In the other pathway, pyruvate is reduced to lactate using NADH, catalyzed by the enzyme LDH (lactate dehydrogenase) (Ke et al., 1993). The role of oxygen and CO_2 in the regulation of these fermentative pathways was extensively studied (Walker et al., 1957; Knee, 1973; Monning, 1983; Kerbel et al. 1988; Ke et al., 1993, 1994a,b, 1995; Kato-Noguchi et al. 1996) and involves the primary metabolic pathways, such as glycolysis, fermentation, TCA cycle and the mitochondrial respiratory chain. In these pathways O_2, as well as CO_2, may affect the enzymatic activity, by changing the rates of degradation and/or synthesis, activation and/or inactivation, substrate and cofactor availability, or a combination of these processes (Watkins, 2000). Despite the effects is related to the concentrations of O_2 and CO_2 and

depends on the commodity (Ke et al., 1993), a common factor that affects the activity of PDC, ADH and LDH is the changes in pH. For instance in 'Bartlett' pears PDC and ADH activation caused by decreased in cytoplasmatic pH (Ke et al., 1994b). Similar results were observed also in 'Chandler' strawberries (Ke et al., 1994a). The effect of CO_2 on pH is usually ascribed to the generation of carbonic acid that reduces intercellular pH (Bown, 1985). The pH changes in the cytoplasm could affect the activity of several key enzymes in situ (Watkins, 2000). In the case of O_2, changes in pH may be associated with stimulation of LDH. Davies et al. (1974) proposed a mechanism to explain how the low oxygen can activates the metabolic pathway involved in the ethanol production: O_2 stress causes an upset in pH regulation in the tissue, diverting carbon to lactate. The formation of lactate causes a drop in the pH, favoring the activity of PDC and the ethanolic fermentation pathway. The decrease in pH, in turn, inhibited LDH activity, ceasing lactate accumulation (Davies, 1980; Roberts, 1989). Following these findings, it is possible that in the present experiment the low oxygen used, even in the presence of CO_2, may have altered the pH regulation, shift most of the pyruvate to the reaction catalyzed by LDH. The contribution of CO_2 in the decrease in pH was probably not relevant. In fact the carbon dioxide concentration applied in the present experiment did not affected the pH, as results in Table 2.6.4.1. Thus the increase in ethanol and acetaldehyde in samples treated with 20 kPa CO_2 in air was probably not related to an acidification but most probably CO_2 might have acted indirectly, increasing the availability of pyruvate. For instance it has been reported that CO_2 inhibit several respiratory enzyme of the Krebs cycle, particularly succinic dehydrogenate (Knee,

1973; Monning, 1983). As consequence the pyruvate can be accumulated, stimulating its degradation in ethanol via PDC and ADH. Since in the present study no enzyme activity, substrate or cofactors, that may be involved in these reactions, were measured, the mechanism proposed above it is an hypothesis and further investigation on this regard must be done to clarify the mechanisms involved during fermentation in fennels.

As reported above, fresh-cut fennel treated with 2 kPa O_2 (in N_2 or with 20 kPa CO_2) did not show any increase in ethanol and acetaldehyde, and this indicates that it well tolerated low oxygen levels. Generally, the lowest recommended oxygen level for CA storage is not less than 2 kPa, however many horticultural commodities, such as apples, can be stored in atmospheres with less than 2 kPa O_2 without detrimental effects (Hoehn et al., 2009). According to Ke and Kader (1992), the tolerance of commodities to low oxygen depends on the internal and the external oxygen concentrations, oxygen consumption rate (that is proportional to the respiration rate of the commodity) and on resistance to O_2 diffusion throughout the plant tissue.

A commodity with a low respiration rate usually tolerates low O_2 better than one with a high rate if the other conditions are the same. Resistance to gas diffusion usually depends on the structure of the dermal system of the commodity: a commodity with a high resistance would require a higher external oxygen concentration to maintain aerobic respiration than one with a low value (Ke and Kader, 1992). The tolerance of fresh-cut fennel to low oxygen levels probably

depends on its low respiration rate but also on the amounts of internal air space of the plant tissue that leads to a consistent decrease in resistance to gas diffusion.

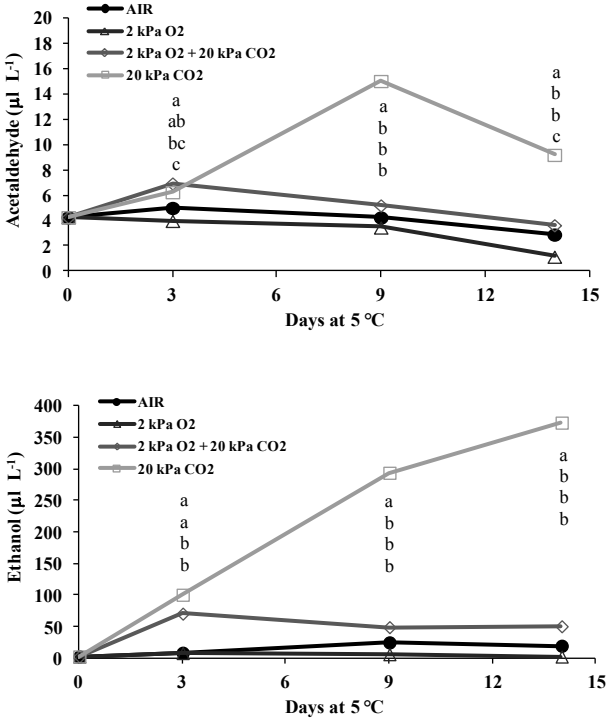

Figure 2.6.4.3 Effect of atmosphere composition on acetaldehyde and ethanol concentrations in fresh-cut fennel stored for 14 days at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Among chemical parameters, total soluble solids and titratable acidity were affected only by treatments while pH, antioxidant activity and vitamin C content (AA, DHAA and AA+DHAA) were influenced only by time of storage (Table 2.6.4.1).

TSS values were significantly higher in fennel slices stored with 2 kPa O_2 (5.8 °Brix) compared to those stored in air (5.3 °Brix) and in 20 kPa CO_2 in air (5.4 °Brix). It could be possible that the slowest respiration rate induced by low oxygen levels delayed the consecutive breakdown of energetic compounds including soluble solids. No significant changes in pH were observed comparing CA treatments despite we expected to have a lower pH in samples stored in CA with high CO_2 since carbon dioxide hydration and the production of HCO_3^- and H^+ may reduce intercellular pH (Bown, 1985). However the dissolution of CO_2 inside the cell could affect differently the cytoplasmatic and the vacuolar pH. For instance Siriphanich et al. (1986) found that cytoplasmatic pH of cut lettuce tissue decreased by about 0.4 pH units and the vacuolar pH decreases by 0.2 when the lettuce was stored for 6 days in 15 or 20 kPa CO_2, however when the pH of these samples were measured in air using a pH meter, they showed a higher pH than lettuce stored in air. A similar phenomenon could have happened in fresh-cut fennel stored in CA with high CO_2. Bown (1985) explained a similar observation as an active regulation of pH by cytoplasm that resulted in an increase in pH when returned to air. Titratable acidity was affected by treatments; in particular the lowest TA was found in fennel slices stored with 20 kPa CO_2 in air or in 2 kPa O_2 (Table 2.6.4.1). These results are in agreement with data reported by Escalona et al. (2005b) that also found the lowest TA values in fresh-cut fennel cv. Clio under highest CO_2 level although in their experiment the increase in carbon dioxide was reached in MAP using non-perforated film. The effect of high CO_2 on the titratable acidity was previously reported on strawberries: Holcroft et al. (1999) observed a reduction of

TA in the juice of strawberry fruits stored in high CO_2, in air or combined with low oxygen, compared to that held in air or with low O_2 alone. Since TA is the sum of acids present as free acids or combined with cations (Ulrich, 1970), it is possible that changes in TA in the present study were correlated to changes in organic acid content. Holcroft et al. (1999) reported that CA enriched with CO_2 (15-20 kPa) can affect the organic acid profile, causing a decrease in TA. Therefore the lower TA values observed in the present trial in samples stored in CA with high CO_2 could be associated to a decrease in organic acid.

Total phenolics content was not influenced by treatments and time, and values ranged between 18.2 and 25.9 mg GAE 100 g^{-1} fw. A significant increase in antioxidant activity was observed during time; values started from 15.2 mg TEAC 100 g^{-1} fw at harvest and reached a mean value of 19.6 mg TEAC 100 g^{-1} fw at the end of the storage, without differences among treatments.

As shown in Table 2.6.4.1, storage time significantly affected the content of vitamin C in terms of AA, DHAA and their total amount. It is well know that fruits and vegetable shown a gradual decrease in AA content during storage (Adisa, 1986; Howard et al., 1999). In addition, since the oxidized form of AA is the DHAA, the decrease of AA over time should be associated to an increase in DHAA. Accordingly, in the present study a gradual decrease in AA as well as an increase in DHAA were observed. In terms of total vitamin C, the amount slightly decreased during storage. Considering that the vitamin C is the sum of AA and DHAA and that the largest share of the total was the AA, changes in total vitamin C during time were mainly influenced by AA more than by DHAA. A significant interaction Time

x Treatment was observed in DHAA content and its trend during storage is showed in Figure 2.6.4.4. One way ANOVA performed at each storage time showed statistical differences only after 14 days at 5 °C when the DHAA significantly increased in fennel slices stored with 20 kPa CO_2, in air or in 2kPa O_2, compared to samples stored in air or in 2 kPa O_2. Previous studies reported that high CO_2 concentration in the storage atmosphere can accelerated vitamin C loss: carbon dioxide may stimulate the oxidation of ascorbic acid by increasing ascorbate peroxidase activity. This enzyme has been proposed to be the major enzyme responsible for enzymatic degradation of AA (Mehlhorn, 1990; Lee et al., 2000, Devlieghere et al., 2002). In addition reducing the O_2 concentration in the storage atmosphere in the presence of high CO_2 had little effect on the vitamin C content (Agar et al., 1997). The mechanism proposed above could explain the higher amount of DHAA observed in samples treated with 20 kPa CO_2 (in air or + 2 kPa O_2) that may indicate a greater AA degradation. However, despite a general decrease in AA over time, no differences among treatments were observed. Agar et al. (1997) proposed an additional ability of CO_2 in the ascorbic acid metabolism: carbon dioxide may inhibit mono- or dehydroascorbic acid reduction to ascorbic acid. According to these authors, it is possible that the presence of high carbon dioxide in CA caused an accumulation of DHAA, inhibiting its reduction to AA.

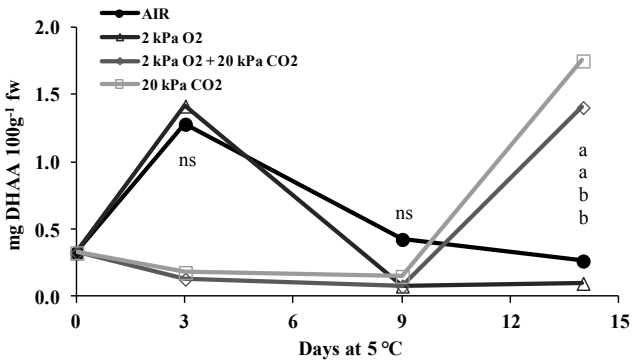

Figure 2.6.4.4 Changes in L-dehydroascorbic acid content in fresh-cut fennel stored under different controlled atmospheres for 14 days at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Both mesophilic bacteria count and the total count of yeasts and moulds were significantly affected by treatment, storage time and their interaction (Table 2.6.4.1). To better understand the effect of treatment at each sampling time, trends of microbial growth during time were reported in Figure 2.6.4.5. The initial count of mesophilic bacteria was 5 log CFU g^{-1} and it was below the limit (5.7 to 6.7 log CFU g^{-1}) prescribed by 'General Directorate for Competition Policy, Consumer Affairs and Fraud Control' for fresh-cut products during processing (DGCCFR 1993).

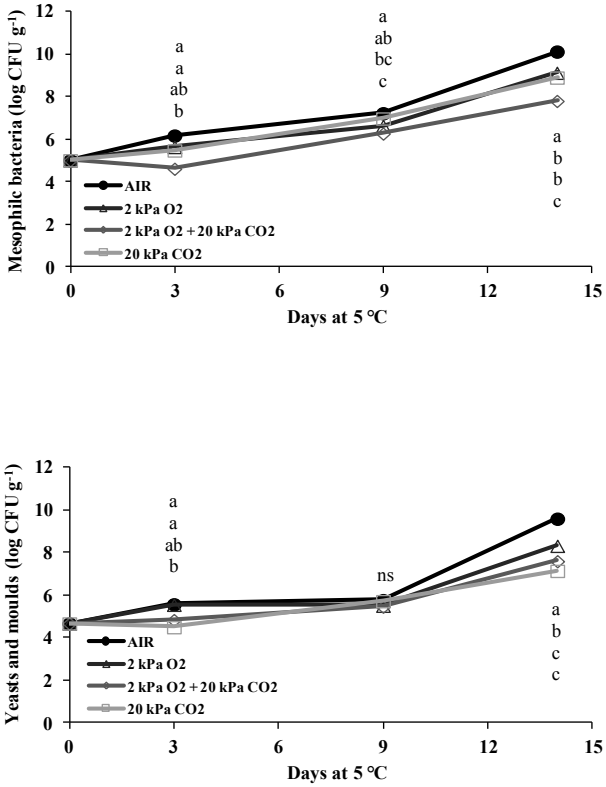

Figure 2.6.4.5 Effect of atmospheric composition on mesophilic bacteria and yeasts and moulds counts in fresh-cut fennel during storage at 5 °C.

During storage, the mesophilic bacteria count significantly increased in all treatments although fennel slices stored in CA with 2 kPa O_2 + 20 kPa CO_2 had significantly lower mesophilic count compared to air control throughout storage duration (Figure 2.6.4.5). After 14 day at 5 °C, fresh-cut fennels stored in 2 kPa O_2 or in 20 kPa CO_2 in air had similar mesophilic count (9.1 and 8.9 log CFU g^{-1} respectively), significantly lower than control (10.1 log CFU g^{-1}) and significantly higher compared to CA treatment with 2 kPa O_2 + 20 kPa CO_2 (7.8 log CFU g^{-1}).

Even if significant differences among treatments were observed at the end of the storage, mesophilic bacteria counts were above the limit (7.7 log CFU g^{-1}) for the consumption of fresh-cut produce as regulated by DGCCFR (1993). On the other hand, taking into account these standards, after 9 day at 5 °C fresh-cut fennel samples were still marketable, regardless of the type of controlled atmosphere applied.

Despite of results reported in Figure 2.6.4.5 for growth of both yeasts and molds, at each sampling day, no colonies of molds were observed therefore the amount reported are probably related to the solely yeasts. The count of yeasts upon processing was 4.7 log CFU g^{-1}, higher compared to the amount detected in the fresh fennel cv. Orion (Escalona et al. 2005a; Escalona et al., 2006). However large differences in microbial counts have been reported between batches of vegetable products (Rico et al., 2007) and the number and type of microorganisms found on fresh produce, and specifically on fresh-cut (minimally processed) products, are highly variable (Zagory, 1999). During the first 9 days of storage the growth of yeasts was very low with statistical differences at day 3 when fennel stored in AIR or in 2 kPa O_2 showed significant higher counts compared to samples stored in 20 kPa CO_2 (in air or + 2 kPa O_2). After 9 days at 5 °C, samples in all treatments had similar counts (5.6 ± 0.1 log CFU g^{-1}) but, at the end of the storage, significant differences were found as slices stored in AIR had a statistically highest yeasts count (9.6 log CFU g^{-1}), followed by samples stored in 2 kPa O_2 (8.3 log CFU g^{-1}), while the lowest count was observed in fresh-cut fennel stored in CA with 20 kPa CO_2 (in air or in 2 kPa O_2). Considering that no differences in yeast count were

found in high CO_2 CA treatments, it possible that the slowdown in growth of yeasts was mainly due to the presence of high CO_2 in the gas mixture. Rattanapanone et al., (2001) found in fresh-cut mango that yeasts increased less under CA conditions (2% O_2 + 10% CO_2) than under storage in air. Similar results were also reported in fresh-cut bell peppers stored for 9 days at 5 °C in which the lowest yeasts count was detected in samples stored under 20 kPa O_2 + 15 kPa CO_2 (Conesa et al., 2007a), and in fresh-cut honeydew melons stored at 5 °C where Qi et al. (1999) reported significantly lower count of yeasts and moulds in CA (2 kPa O_2 + 10 kPa CO_2) compared to samples stored in air. Considering the microbiological quality of fresh-cut fennel stored in CA treatments, the gas mixture with 2 kPa O_2 + 20 kPa CO_2 showed best result in delay growth of both mesophilic bacteria and yeasts during storage compared to treatments in AIR and with low oxygen. Many authors reported the effect of carbon dioxide in arresting or slowing down microbial growth (Daniel et al., 1985; Zagory, 1999; Brown, 1922). For instance Portela et al. (1997) had similar results in cantaloupe melon cylinders (cv. Durango) stored in different CA conditions for 9 and 15 day at 10 and 5 °C respectively: low oxygen atmospheres (1 or 3 %) had little effect on microbial growth in fresh-cut cantaloupe melon, but a combination of low oxygen (3%) and high CO_2 (7.5 or 15%) was effective in controlling microbial growth and decay at both storage temperature. Similarly Berrang et al. (1990) reported that enriched CO_2 atmospheres had a significant inhibitory effect on the growth of aerobic micro-organisms on broccoli kept at 4°C. The antimicrobial properties of high CO_2 concentrations are mostly due to a reduction of pH and interference with the cellular metabolism (Brackett, 1997;

Faber et al., 2003). However in our study the effect of gas composition on pH values was not relevant. Similarly, Babic et al. (1996) reported no effect of CA (0.8% O_2 and 0.8% O_2 + 10% CO_2) on pH in fresh-cut spinach suggesting that the inhibitory effect of CA on microorganisms was not due to an acidification of the microbial environment, but probably to a decreased oxygen availability. However these authors used very low oxygen concentration (0.8%) so it is possible that the very low oxygen availability had a major effect compared to that of CO_2. In the present experiment, comparing microbial quality of fresh-cut fennel stored in 20 kPa CO_2 (in air and + 2 kPa O_2), a significant effect of high CO_2 on the growth of yeasts was observed, while in the case of mesophilic bacteria better results were obtained using the gas mixture 2 kPaO_2 + 20 kPa CO_2 and it could be due to a synergic effect of low oxygen and high CO_2 concentrations.

A principal component analysis (PCA) was performed using average values of three replicates for each treatment (2 kPa O_2, 20 kPa CO_2 in air, 2 kPa O_2 + 20 kPa CO_2 and AIR) at each sampling day (0, 3, 9 and 14) of sensorial (appearance score, aroma, crunchiness, stem browning, sheath browning, dehydration, flavour, sweetness, overall quality), physical (weight loss, hue angle in both stem and sheath), chemical (TTS, pH, TA, total phenolic content, antioxidant activity, total vitamin C, ethanol, acetaldehyde) and microbiological (mesophilic bacteria, yeasts and moulds) parameters. The PCA conducted on these data showed that the model accounted for 74.7% of the total variance of experimental data analyzed, with PC1 and PC2 explaining 59.6% and 15.1% of the variance, respectively. The loading of

the attributes analyzed on the PC1-PC2 plane is showed in Figure 2.6.4.6. A significant clustering of the variables, with the widest differences on PC1, was observed, denoting a high correlation among several quality attributes of fresh-cut fennels. The sensorial attributes correlated with higher quality of fresh-cut fennels (sweetness, flavour, crunchiness, aroma, appearance score and overall quality) were positioned close together on the left hand side of PC1 whereas on the right side were grouped the attributes in which high scores indicate a loss of sensorial quality (stem and sheath browning, dehydration). In addition, also weight loss and microbial counts (mesophilic bacteria and yeasts and moulds) that are correlated with low quality, were positioned on the same side. The weight loss and dehydration were very close in the PC1-PC2 plane, indicating, as expected, an elevated correlation between the loss of water content and the sensorial evaluation of dehydration. The obtained results confirmed that the main source of data variation (over the PC1) was the time of storage; the sensorial attributes placed on the right hand side described the most relevant characteristics of the fennels as just cut whereas the loss of weight and of the sensorial and microbiological quality of the product better represented fresh-cut fennels at the end of the storage. Total phenolics, antioxidant activity and total soluble solids were also placed on the right hand side of PC1 indicating that these parameters increased with the storage duration. The increase in phenolics is desirable from the nutritional point of view since they act as antioxidants; in fact phenolics and antioxidant activity were not distant from each other in the PC1-PC2 plan. On the other hand, as showed in Figure 2.6.4.6, phenolics content was very close to stem browning indicating that

these attributes were highly correlated. In fact, besides the health-promoting properties of phenolics, these compounds are substrates for oxidative enzymatic and non-enzymatic browning reactions, therefore increasing the content of phenolics, browning of cut-surfaces increases. As expected an inverse relationship between the positions of sensorial evaluation of the browning and hue angle, in both stem and sheath, were found: hue angle decreased when the browning occurred. Ethanol and acetaldehyde contents influenced the PC model, but with the lowest projection on the PC1 and, on the contrary, the highest on the PC2. These parameters were placed close together in the PC1-PC2 plane because both are products of fermentative reaction. Among quality attributes analyzed, vitamin C and pH had low weight in the PC model.

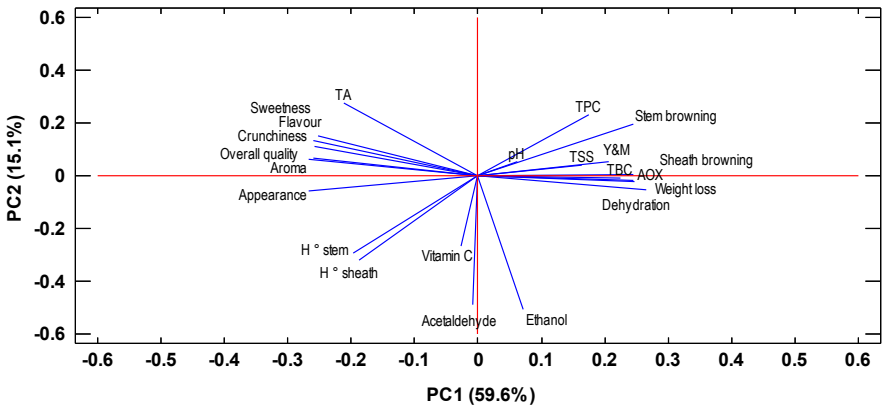

Figure 2.6.4.6 Loading factors describing the relationship among quality attributes of fresh-cut fennel stored under different controller atmosphere for 14 days at 5 °C.

Analyzing the scores of the treatments at each sampling day in the PC1-PC2 plane (Figure 2.6.4.7), in general samples moved from the left hand side at time zero, to the right one at the end of the storage. In particular fresh-cut fennel held in air or with 2 kPa O_2 moved on PC1 axes, from the left to the right hand side, faster that samples stored in CA with high CO_2 level. The position of samples held in 20 kPa CO_2 in air at 9 and 14 days was in the negative part of the PC2, far away than others CA treatments respect to the negative quality attributes, indicating that fresh-cut fennel stored in 20 kPa CO_2 in air better maintained the quality attributes, although these samples had an higher correlation with ethanol and acetaldehyde contents compared to others treatments.

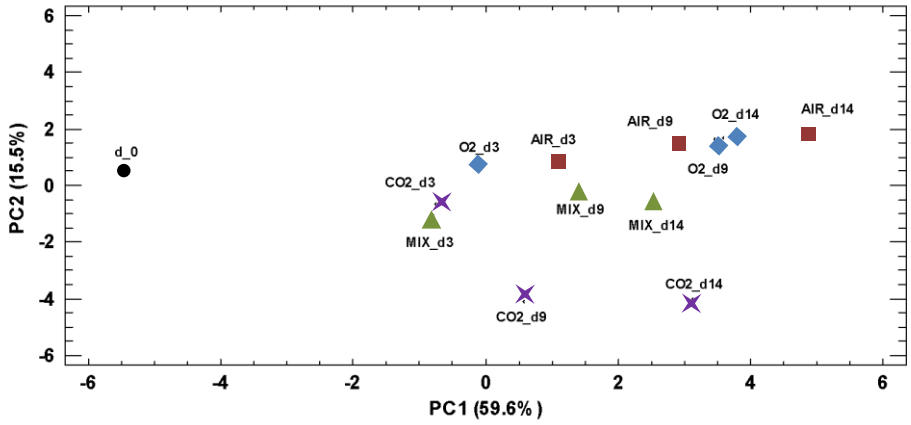

Figure 2.6.4.7 Score plot describing the relationship among treatments at each sampling day of fresh-cut fennel stored under different controller atmosphere for 14 days at 5 °C.

(CO2 = 20 kPa CO_2 in air; MIX = 2 kPa O_2 + 20 kPa CO_2; O2 = 2 kPa O_2; AIR = control in air; d=sampling day)

Starting from results of PCA, the scores of the PC1 for each treatment were fitted by the conventional zero- and first-order kinetics, the last one with and without data normalization. Since the scores showed an exponential increase over time, data were normalized between 0 and 1, referring to the lowest and the highest value. This preprocessing improved the fitting of the experimental observations as shown Table 2.6.4.3 which include the kinetic parameters and the coefficient of determination (r^2) of the fitting. From the results emerged that the exponential model of normalized data better explained the PC1 score changes during storage, showing a correlation coefficient higher than 0.97. Figure 2.6.4.8 shows the fitting of PC1 scores for each CA treatment over time. Using zero- and first-order kinetics, r^2 values in the range of 0.81-0.87 and 0.72-0.80, respectively, were obtained. Comparing the multivariate rate constants (k) of the exponential model, which best fitted the data, it can be observed that 20 kPa CO_2 in air treatment showed the lowest k value (-0.20) indicating that, taking into account all the quality parameters analyzed in the PCA, the loss of quality of fresh-cut fennel over time was slower in this CA condition, compared to the other treatments. In the meantime the highest degradation was observed in fresh-cut fennel stored in 2 kPa O_2 that had the highest k value (-0.32).

Table 2.6.4.3 Kinetic regression parameters of the PC1 scores as a function of time. Data related to fresh-cut fennel stored under different controlled atmosphere for 14 days at 5 °C.

Zero order kinetic, PC1 score(t) = $a_0 + kt$		
Treatments	**k**	**r^2**
2 kPa O2	0.61	0.81
2 kPa O2 + 20 kPa CO2	0.52	0.84
20 kPa CO2	0.54	0.87
AIR	0.65	0.81
First order kinetic, PC1 score(t) = $a_0 * \exp^{(-kt)}$		
Treatments	**k**	**r^2**
2 kPa O2	0.07	0.72
2 kPa O2 + 20 kPa CO2	0.06	0.75
20 kPa CO2	0.07	0.78
AIR	0.07	0.70
Normalized first order kinetic, PC1 score(t) = 1 - $\exp^{(k*t)}$		
Treatments	**k**	**r^2**
2 kPa O2	-0.32	0.98
2 kPa O2 + 20 kPa CO2	-0.27	0.99
20 kPa CO2	-0.20	0.97
AIR	-0.29	0.99

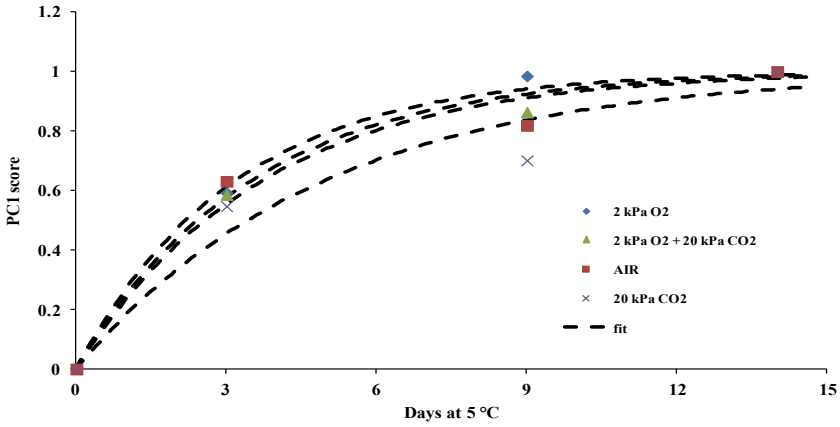

Figure 2.6.4.8 Changes of PC1 score over time for fresh-cut fennel stored under different controlled atmospheres at 5 °C.

The final goal of MALST is to use the chart in Figure 2.6.4.8 to estimate the shelf-life by finding a cut-off limit obtained by inserting, for each quality parameter, its critical value for the product marketability. This final step, normally applied for finished products (packaged), was not calculated in this study, since the general objective was to compare the effect of different treatments on the overall quality degradation.

2.6.5 Conclusion

The purpose of the present study was to understand the effects on fresh-cut fennel of storage atmosphere modification including low oxygen, high CO_2, and their combinations, in order to identify best suitable gas mixture to extend its shelf-life. Results showed that the presence of CO_2 in the gas mixture significantly delayed the browning of fresh-cut fennel, while the O_2 concentrations in the CO_2 enriched atmosphere slightly affected the visual quality. Best results were obtained using an atmosphere of air enriched with 20 kPa CO_2 that was effective to preserve visual appearance of fresh-cut fennels stored at 5 °C for 14 days, delaying the occurrence of browning on the cut surfaces, and these results were also confirmed by the multivariate analysis. However, an atmosphere of 20 kPa CO_2 in air is not likely to be obtained in modified atmosphere packaging (MAP) where the CO_2 accumulation cannot be unrelated to oxygen consumption. Therefore for the application of MAP technology, gas atmospheres combining 20 kPa CO_2 to low oxygen could be used

instead of atmosphere of 20 kPa CO_2 in air, also considering that oxygen level was not critical for the quality of fresh-cut fennels.

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2.7 DESIGN A MODIFIED ATMOSPHERE PACKAGING FOR FRESH-CUT FENNEL

2.7.1 Abstract

The aim of the following experiments was to design a modified atmosphere packaging (MAP) in bags in order to reach the optimal gas composition (2-5 kPa O_2 + 20 kPa CO_2), as resulted from previous studies performed in CA conditions (Experiment 2.6), In the first experiment fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Tiepolo*) were cut, dipped in EtOH 0.5% as antibrowning agents (Experiment 2.5), and kept in air or packed in polypropylene film (PP) without (NMP) or with one (MP1) or two (MP2) layers of microperforation, flushing an atmosphere of 5 kPa O_2 + 20 kPa CO_2 in nitrogen inside the bags. All samples were stored at 5 °C for 10 days, evaluating the gas changes over storage time. PP NMP and PP MP2 were discarded since rapid anoxic conditions (in PP NMP) and a too high gas exchanges (in PP MP2) occurred already after 24 h of storage, while in PP MP1 samples a steady state of about 12 kPa O_2 and 10 kPa CO_2 was rapidly reached. Thus PP MP1 samples and fresh-cut fennels stored in air (CTRL) were evaluated after 3, 8 and 10 days at 5 °C for sensorial (appearance score, stem and sheath browning score), physical (stem and sheath color, weight loss) and chemical (TSS, phenolic, antioxidant activity, vitamin C, ethanol, acetaldehyde contents) attributes. Despite not reaching the target gas concentrations PP MP1 resulted effective in reducing browning of the fennel cut-surfaces, to better maintain the nutritional values and to avoid the loss of

weight compared to control in air. In the second experiment a passive MAP was used testing 2 different plastic material (PP MP1 and PP+PA MP1) in order to optimize packaging design. Better results were obtained with PP+PA MP1 that allowed to reach the desired gas concentration inside the bags.

In the third experiment fennel heads cv. *Apollo* were processed as described above and samples of about 150 and 200 g were closed in PP MP1 and PP+PA MP1 respectively, flushing an initial atmosphere of 5 kPa O_2 + 20 kPa CO_2 in nitrogen inside the bags. CTRL samples of about 150 g were kept in air. Changes in gas composition in PP MP1 and PP+PA MP1 samples were monitored over time. Samples were evaluated initially and after 3, 7 and 13 days of storage at 5 °C for the same attributes described in the first experiment. In addition, HPLC determination of sugars and organic acids as well as microbiological quality (mesophilic, psychrophilic, lactic acid bacteria, yeasts and moulds counts) were estimated. The suitable gas composition (5 kPa O_2 + 20 kPa CO_2) were maintained in PP+PA MP1 samples over time. These gas compositions proved to be effective in delaying browning in both stem and sheath cut-surfaces, and in controlling the mesophilic and psychrophilic growth as well as enterobacteriaceae contamination up to 7 days compared to CTRL. In terms of nutritional quality, a loss of vitamin C occurred in all treatments while no changes over time were observed for phenolic compounds, sugars and organic acids. Therefore, based on results of the present experiments, packaging 200-250 g (depending on the respiration rate) of fennel slices, dipped in ethanol 0.5%, in PP+PA MP1 bags (15 x 20 cm) with initial gas composition of 5 kPa O_2 and 20 kPa CO_2 is effective in maintaining a very good visual quality,

without main nutritional losses. In addition shelf-life in all the tested conditions, was estimated applying the Multivariate accelerate shelf-life test (MASLT). Based on the model obtained, the shelf-life for stored fresh-cut fennels was 9.7, 12.2, and 24.2 days for air, PP MP1 and PP+PA MP1 conditions, respectively.

2.7.2 EXPERIMENT 1

2.7.2.1 Objective

The main objective of this experiment was to design a MAP to maintain into the bags a gas concentration as close as possible to 5 kPa O_2 + 20 kPa CO_2, which, from previous experiment in CA (Experiment 2.6), resulted the most suitable for preserving quality of fresh-cut fennels. A secondary objective of thesis experiment was to estimate the shelf-life of fresh-cut fennel in these conditions. Respect to the other studies available in literature where fresh-cut fennel was packed in sealed plastic trays, MAP in bags may be more flexible to be used by processors (in term of machinery setting conditions as material, and dimensions), and less impacting on the environment.

2.7.2.2 Experimental setup

Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Tiepolo*) were harvested on March 2015 in Puglia (Italy), transported in cold conditions to the Postharvest laboratory of the University of Foggia and stored at 0 °C until processing. After trimming operations, heads were washed in chlorine solution (0.01% v/v) for 2 min, rinsed in tap water for 1 min and dried. Each fennel head

was then cut into slices of approximately 1 cm thickness, immersed for 2 min in 0.5% ethanol solution and then dried with 2 layers of cheesecloth. Samples of approximately 150 g were packaged in active modified atmosphere (5 kPa O_2 + 20 kPa CO_2 in nitrogen) using a packaging machine (Mod. T520, Tecnovac, Grassobbio, BG, Italy), in polypropylene (PP) bags (dimensions 15 × 20 cm), with one (MP1) or two (MP2) layers of laser microperforation, or without microperforation (NMP). The characteristics of each film type, measured in previous experimental conditions (in term of gas differential pressure at the equilibrium) are reported in Table 2.7.2.2.1.

Table 2.7.2.2.1 Characteristics of polypropylene film used for packaging.

	Thickness (my)	Diameter of holes	Number of holes/m^2	OTR (ml m^{-2} day^{-1})	CO2TR (ml m^{-2} day^{-1})	β (CO2TR/OTR)
PP NMP	30	-	0			
PP MP1	30	60	84	2500	2800	1.12
PP MP2	30	60	168	4650	4650	1

Additional replicates of the same weight were placed in macro-perforated polyethylene clam-packs (119 x 189 x 90 mm; capacity 500 g; CL1/90 Carton Pack®) and were used as control (CTRL), while 3 samples were used for initial determinations. All samples (3 replicates × each treatment × sampling time) were stored at 5 °C and analyzed after 3, 8 and 10 days. The quality attributes evaluated during this experiment were:

- headspace gas composition;

- sensorial attributes (visual appearance, stem and sheath browning);
- stem and sheath color;
- weight loss;
- total soluble solid (TSS);
- total phenols content;
- antioxidant activity;
- vitamin C (total, L-ascorbic and L-dehydroascorbic acid);
- acetaldehyde and ethanol content.

2.7.2.3 Results and discussion

The evolution of gas composition within the package is showed in Figure 2.7.2.3.1. In the PP NMP the absence of microperforation determined anoxic conditions (O_2 < 0.5 kPa) already after 24 h of storage because the oxygen inside the bags was quickly consumed during the respiration process of the product. At the same time the CO_2, after a slight decrease in the first 24 h, gradually accumulated up to 37.9 kPa after 10 days at 5 °C. Fresh-cut fennels stored in PP MP1 showed an increase in O_2 and a decrease in CO_2 reaching the steady state already after 24 h. In these samples the levels of oxygen and carbon dioxide remained stable up to 9 days with mean values of 11.7 ± 1.1 and 10.3 ± 0.4 kPa respectively, and changed slightly at day 10, most probably due to a decrease in respiration activity of the product. Also in PP MP2 bags the steady state was reached after 24 h of storage with of 14.7 ± 0.5 kPa of O_2 and at 6.8 ± 0.3 kPa CO_2.

Since the established gas conditions were not suitable for fresh-cut fennel, the samples packaged in PP NMP and PP MP2 were discarded and only samples packaged in PP MP1 were analyzed for quality attributes, and compared with CTRL samples stored in air.

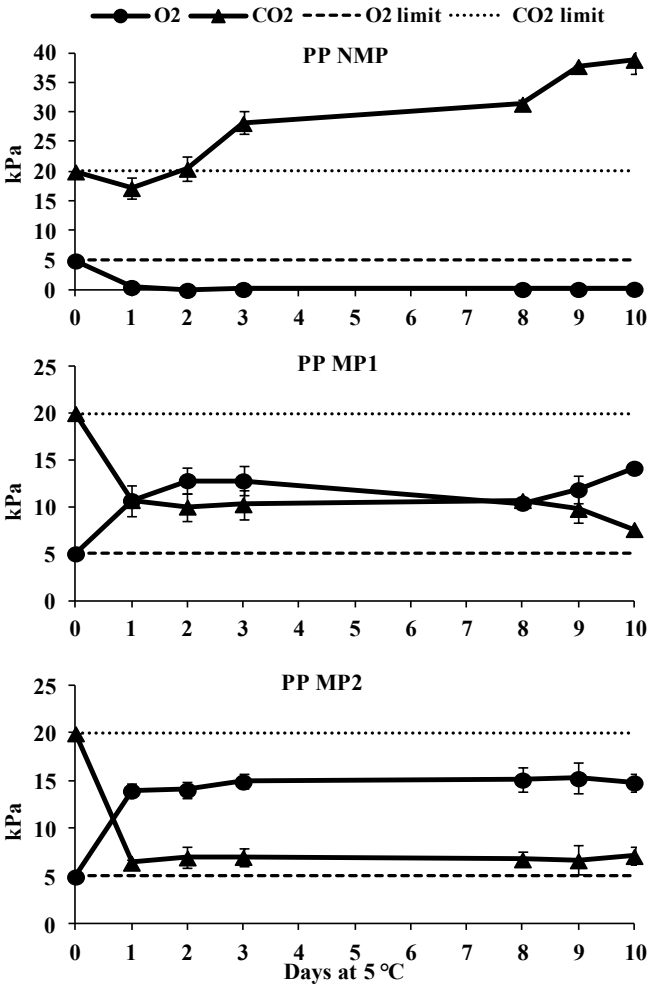

Figure 2.7.2.3.1 Oxygen and carbon dioxide concentrations within packages. Mean values of 3 replicates ± STD.

The effect of treatments (PP MP1 and CTRL), time of storage and their interaction on quality parameters of fresh-cut fennel cv. *Tiepolo* are showed in Table 2.7.2.3.1. Treatment significantly affected most of the physical and sensorial parameters analyzed, except for the browning, a* value and hue angle of the sheath cut-surfaces. As for chemical parameters total vitamin C, L-dehydroascorbic acid, L-ascorbic acid and acetaldehyde contents were not influenced by the treatment. Time of storage significantly influenced all the sensorial and physical attributes, except the lightness (L*) of the sheath cut-surface and total phenolic content among the chemical. The interaction time x treatment was statistically significant for the development of browning in the stem part of the slices, weight loss, a* value of the stem and L* value of the sheath, as well as for total soluble solids and total phenolic contents.

Table 2.7.2.3.1 Effect of treatments (PP MP1 and CTRL), storage time and their interaction on quality parameters of fresh-cut fennel during storage at 5 °C. Data are mean values of 9 samples (3 replicates x 3 storage time).

Parameters	PP MP1	CTRL	Treatment	Time	Treatment X Time
Sensorial attributes					
Visual appearance	3.5	2.4	****	****	ns
Stem browning	2.2	3.3	****	****	*
Sheath browning	1.2	1.2	ns	****	ns
Physical attributes					
Weight loss (%)	0.1	10.3	****	****	****
Stem color					
L*	86.5	82.2	****	**	ns
a*	-0.4	1.0	****	****	*
b*	15.2	16.7	***	****	ns
Chroma	15.2	16.8	****	****	ns
Hue angle	91.7	86.6	****	****	ns
Sheath color					
L*	85.3	83.8	***	ns	*
a*	-2.7	-2.8	ns	**	ns
b*	12.7	14.1	**	**	ns
Chroma	13.0	14.4	**	*	ns
Hue angle	102.3	101.3	ns	***	ns
Chemical attributes					
Total soluble solid (°Brix)	5.6	6.4	****	ns	**
Total phenol content (mg GAE 100 g^{-1} fw)	22.3	28.5	****	****	****
Antioxidant activity (mg TEAC 100 g^{-1} fw)	15.5	17.9	*	ns	ns
Ascorbic acid (mg 100 g^{-1} fw)	10.9	11.1	ns	ns	ns
L-dehydroascorbic acid (mg 100 g^{-1} fw)	4.9	6.3	ns	ns	ns
Vitamin C (mg 100 g^{-1} fw)	15.8	17.4	ns	ns	ns
Ethanol (ul L^{-1})	20.1	6.1	*	ns	ns
Acetaldehyde (ul L^{-1})	3.3	2.3	ns	ns	ns

Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). Different letters indicate statistical differences within treatments, according to the Tukey's test ($p \leq 0.05$).

Even though the atmosphere at the equilibrium was not the target one (higher oxygen level), the effect of the atmosphere modification and mainly of the accumulation of the CO_2, positively affected the visual appearance of the MAP stored sample, if compared to CTRL (Figure 2.7.2.3.2). In particular the use of PP MP1 allowed to maintain the marketability of the product up to 10 days at 5 °C whereas the visual quality of fennels stored in air (CTRL) rapidly decreased during storage, reaching a mean value of 1.9 after 10 days at 5 °C. Considering that the browning in the sheath cut-surface was very mild (average value of 1.2) and that no differences were observed between treatments, the main factor that affected visual quality was probably the browning of the stem, in accordance with Albenzio et al. (1998) who considered enzymatic browning as the main cause of postharvest deterioration of minimally processed fennels. Treatment in MAP significantly delayed the browning development in fennel stems during storage (Figure 2.7.2.3.3); in fact only a moderate browning score was assigned after 10 days at 5 °C (2.8). Comparing treatments at each sampling day, the level of browning of the stem was significantly higher in fresh-cut fennel stored in air (CTRL). In these samples browning rapidly increased already in the first 3 days of storage, moving from 1.3 at time zero to 2.7, and then continued to slowly increase up to day 8 with a final value of 3.5 which was maintained up to the end of the storage. Also results described by Escalona et al. (2005b) on sliced fennels stored in MAP showed that the higher visual appearance scores were correlated to the lower level of browning of the cut surface, even though they did not distinguish between stem and sheets. In contrast Escalona et al. (2005a) did not observe a significant effect of MAP on

appearance and browning of fennel dices after 14 days at 0 °C but the same authors suggested that the oxygen levels reached was not low enough to prevent browning. The positive effect of MAP in inhibiting browning reaction, maintaining visual appearance, has been extensively reported in fresh-cut fruits (Aguayo et al., 2003; Alique et al., 2003; Martínez-Ferrer et al., 2002) and vegetables (Aguayo et al., 2004; Baskaran et al., 2001; Fernández-León et al., 2013; Serrano et al., 2006), mainly due to the O_2 reduction and/or elevated CO_2 accumulation which can delay the oxidation of phenolic compounds, caused by the polyphenol oxidases (i.e. PPO), and reduce the availability of reaction substrates, acting on their biosynthesis (Gorny, 1997).

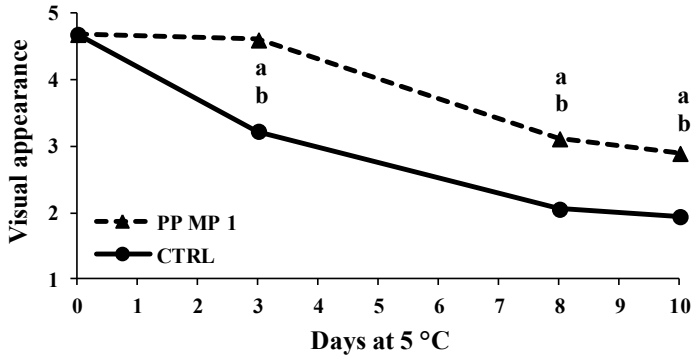

Figure 2.7.2.3.2 Visual appearance and stem browning scores of fresh-cut fennel during storage at 5 °C. Within the same storage evaluation, different letters indicate statistical differences. Scale: 5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

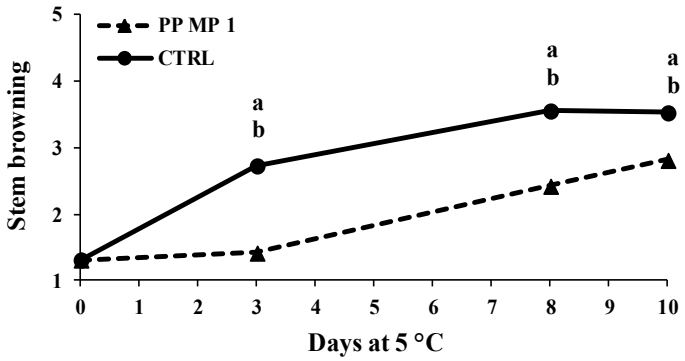

Figure 2.7.2.3.3 Visual appearance and stem browning scores of fresh-cut fennel during storage at 5 °C. Within the same storage evaluation, different letters indicate statistical differences. Scale : 1= absence of browning, 3= slight browning, 5= completely brown.

According to results of sensorial evaluation of stem browning, color data related to the stem cut-surface revealed a significant increase in browning in CTRL samples compared to fresh-cut fennels stored in MAP, as indicated by significantly lower levels of lightness (L*), the higher values of a*, b* and chroma, and the lower hue angle compared to samples in MAP (Table 2.7.2.3.1). Changes in L*, a* and hue angle values over time are shown in Figure 2.7.2.3.4. The lightness was almost stable in PP MP1 samples, only showing a slight decrease over time, whereas in CTRL samples, L* started to decrease already after 3 days of storage and then continued to slowly decrease up to day 10. A significant interaction between treatment and time of storage was present for a* value. As showed in Figure 2.7.2.3.4 the increase in a* value was much faster and bigger in CTRL compared to MAP samples: in particular a* value increased from negative to positive

(disappearance of green component) for CTRL samples whereas in packaged samples at the end of the storage a* value did not reach above 0. A reduction in hue angle was also observed in both treatments, significantly lower in MAP samples compared to CTRL (Figure 2.7.2.3.4).

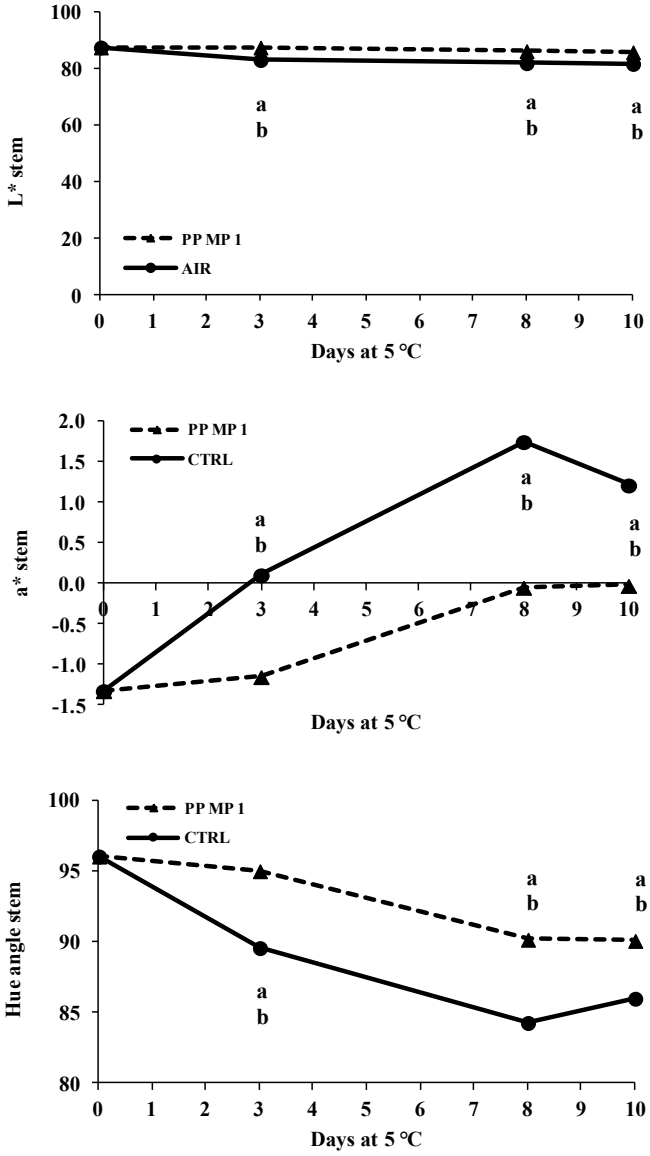

Figure 2.7.2.3.4 Changes in L*, a* and hue angle on stem part of fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences.

Despite not having observed differences between treatments for visual evaluation on the sheath part of fennel slices, the color analysis has highlighted some differences. In particular CTRL samples had significant lower level of L* and higher b* and chroma values compared to MAP samples, whereas a* value was similar between treatments (Table 2.7.2.3.1). The lightness (L*) slightly decreased in CTRL samples over time, while it remained almost constant and significantly higher than CTRL for PP MP1 samples until day 8. No significant differences in L* values between treatments were observed at the end of the storage when a very slight loss of lightness also occurred in samples stored in MAP (Figure 2.7.2.3.5). As reported above, in both treatments panelists observed a very slight browning on the sheath cut-surface over time and it was probably due to a yellowing that occurred either in MAP or in CTRL samples, as demonstrated by a decrease in hue angle that changed from light green to yellow (Figure 2.7.2.3.5).

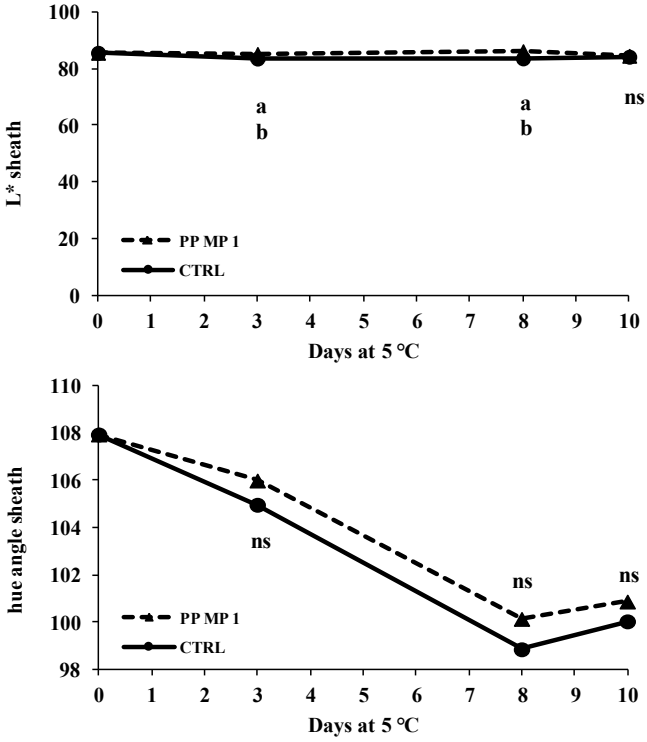

Figure 2.7.2.3.5. Changes in L* and hue angle values on sheath part of fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences.

Escalona et al. (2005a) noticed that only the duration of storage and not the treatment in MAP affected the color of diced fennel stored for 14 days at 0 °C, though in their experiment O_2 and CO_2 concentrations applied were respectively higher and lower compared to that of samples in PP MP1 bags in this experiment. In addition, in diced pieces the level of wounding is much higher than in the slices. On fennel slices, the atmospheres with 16-18 kPa O_2 and 2-4 kPa CO_2 generated

within perforated packages at 0 and 5 °C, or with 1.5-2 kPa O_2 + 18-20 kPa CO_2 generated in unperforated packages at 5 °C, did not inhibit browning on the cut surfaces, while an atmosphere with 4-6 kPa O_2 + 10-14 kPa CO_2 at 0 °C helped to maintain better sensorial quality (Escalona et al., 2005b). Moreover a in a another study a controlled atmosphere with 5 kPa O_2 + 15 kPa CO_2 delayed browning of fennel slices for 14 days at 5 °C (Escalona et al., 2006). These last results from Escalona et al. (2005b; 2006), suggested that the gas composition with low oxygen and moderate CO_2 concentrations was effective in delaying browning. Comparing these results with data of the present experiment, even if O_2 and CO_2 concentrations reached inside PP MP1 bags were respectively higher and lower compared to the optimal gas concentrations suggested by Escalona et al. (2005b; 2006), and by ours previous findings, browning was sufficiently delayed, probably also thanks to the use of the pretreatment with 0.5% ethanol. Further comparisons with appearance and color data reported by Escalona et al. (2005a,b; 2006) may be inaccurate since these authors evaluated browning of the fennel dices/slices without any distinction between stem and sheath parts.

Separate color evaluation between butt end cut zone and external leaves was performed on whole fennel cv. Orion stored in MAP (by Escalona et al., 2004). In accordance with results of the present study, the authors observed that on the butt-end cut zone changes in color were particular more intense in CTRL compared with MAP samples, while they did not find significant changes in color parameters on the external leaves.

A significant loss of weight was observed only in CTRL samples, that lost about 15% of the initial weight after 10 days of storage, while in fennel slices stored in MAP the weight remained almost the same during storage (Figure 2.7.2.3.6). This because, although fresh-cut products are highly susceptible to weight loss because of the high cut surfaces exposed to air, the use of packaging represent a barrier to vapor diffusion which allows to maintain an adequate relative humidity within the package so tissue dehydration is limited (Zagory et al., 1988; Watada et al., 1999).

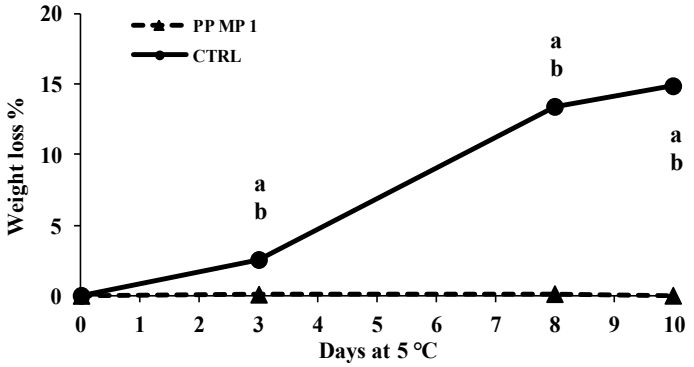

Figure 2.7.2.3.6. Weight loss in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences.

The significant loss of weight in CTRL samples could have influenced other parameters, generally inducing a concentration of internal constituents. For example the content of TSS remained almost constant over time in fresh-cut fennel stored in MAP (mean value 5.6), while an increase was observed in CTRL samples (mean value 6.4) (Table 2.7.2.3.1). Generally TSS values decrease during time because

sugars and organic acid are used for the production of energy during the respiration process. In the case of fresh-cut fennel stored in PP MP1, the use of MAP could have delayed this process, allowing to preserve the content of TSS. Zagory et al. (1988) also reported that one of the primary effects of modified atmosphere is a lower rate of respiration, which reduces the rate of substrate depletion.

A highly significant ($p \leq 0.0001$) effect of treatment, time of storage as well as of their interaction were found in the content of total phenolic compounds (Table 2.7.2.3.1); changes over time for this attribute are shown in Figure 2.7.2.3.7. The amount of phenolic compounds slightly increased from 20.6 ± 1.1 GAE $100g^{-1}$ fw at time 0 to 22.2 ± 0.7 and 23 ± 0.6 GAE $100g^{-1}$ fw after 3 days in PP MP1 and CTRL respectively, without significant differences between treatments. In CTRL samples the content of phenolics kept increasing up to 31.5 ± 0.2 GAE $100g^{-1}$ fw at day 8 and then remained relatively constant until the end of the storage while samples in MAP did not show any significant increase until day 10. The initial increase in phenolic compounds could be caused by cutting: in fact one of the consequences of mechanical injury due to tissue wounding is the induction of secondary product synthesis, including a variety of phenolic compounds (Saltveit, 1997; Saltveit, 2000; Garcia et al., 2002). In CTRL samples, the further increase in phenolic compounds over time could be a result of weight loss, that caused solutes concentration.

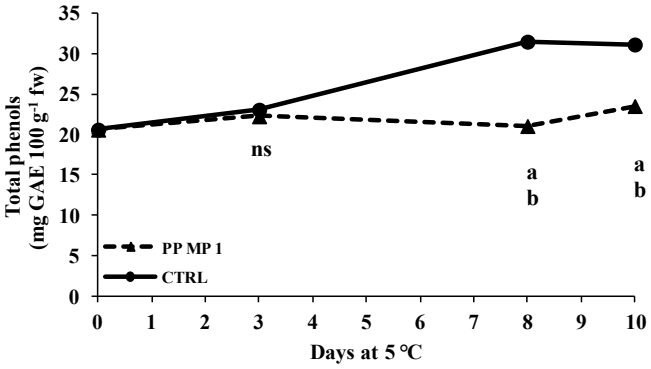

Figure 2.7.2.3.7 Changes in total phenols in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences.

As reported in Table 2.7.2.3.1, also antioxidant activity was significantly affected by treatment. In accordance with results on phenolic content, antioxidant activity was higher in CTRL compared to MAP samples. A correlation between phenolic content and antioxidant activity is commonly reported since phenolics are the compounds with major relevance in the total antioxidant capacity of fruits and vegetable (Jacobo-Velázquez et al., 2009). Vitamin C content is showed in Table 2.7.2.3.1 as amount of AA, DHA and their sum: no effect of time of storage and treatment was observed for these parameters.

As showed in Figure 2.7.2.3.8, the content of ethanol decreased in CTRL samples during storage while fennel slices stored in MAP had a steady level of ethanol, significantly higher than CTRL samples only at last sampling day. The production of ethanol and acetaldehyde in plant is not necessarily a consequence of a reduction of oxygen availability, but it could be related to an alteration of respiratory

metabolism occurred under stress conditions (Kimmerer et al. 1982). Therefore it is possible that the initial level of ethanol detected in this experiment was a stress response to cutting operations. In CTRL samples, ethanol decreased over time, whereas in MAP samples its content remained almost constant during storage, probably because of the production by the tissues in the presence of high CO_2 inside the bags. These results are in accordance with Mateos et al. (1993) and Forney et al. (2009) regarding the possible effect of CO_2 on the accumulation of fermentative products in fruits and vegetables, and furthermore confirmed what was previously observed in CA experiments reported in this dissertation (Experiment 2.6). On the other hand the level of acetaldehyde detected in this study was very low and no significant changes were observed over time and in relation to treatments.(Table 2.7.2.3.1).

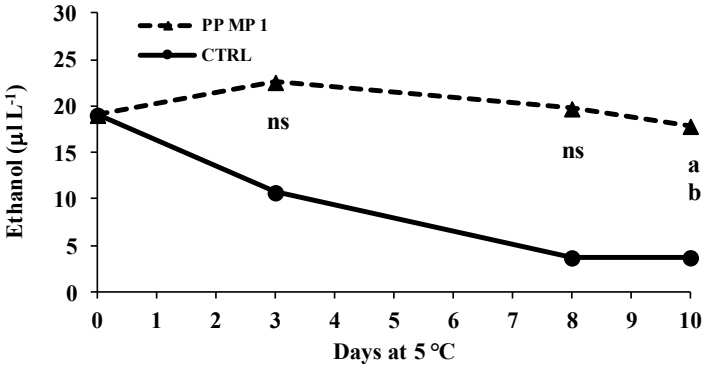

Figure 2.7.2.3.8. Changes in ethanol content in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences.

2.7.2.4 Conclusions

Starting from an initial gas composition of 5 kPa O_2 +20 kPa CO_2, a steady state of about 12 kPa O_2 and 10 kPa CO_2 was reached in PP MP1 samples. Despite this atmosphere was not the target one (2-5 kPa O_2 + 20 kPa CO_2), the positive effect of MAP was confirmed: fennel slices stored in PP MP1 maintained the marketability up to ten days at 5 °C. In addition MAP preserved not only the color of fresh fennel slices but also most of the chemical attributes analyzed. Considering the results obtained from this first experiment in MAP and the desirable gas mixture inside the bags, packaging design needs to be optimized.

2.7.3 EXPERIMENT 2

2.7.3.1 Objective

Based on results of previous experiment we introduced a different material with higher gas barrier proprieties optimized also for weight using the following formula:

Desired Gas Transmission Rate (GTR) to O_2 and CO_2, respectively OTR and CO2TR, was calculated per mil of thickness (1 mil = 25.4 μm) of plastic material using the following formula:

$$GTR = \frac{W * RR}{A * |\%G_{atm} - \%G_{pkg}|}$$

where W = product weight (kg), RR = respiration rate (ml kg^{-1} day^{-1}); A = packaging surface (m^2); $\%G_{atm}$ = percentage of the gas in the atmosphere; $\%G_{pkg}$ =

desired percentage of the gas in the packaging (5 kPa O_2 and 20 kPa of CO_2, as previously defined).

The objective of this experiment was to monitor gas concentrations over storage and confirm the hypothesis for 3 different packaging design.

2.7.3.2 Experimental setup

Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Tiepolo*) were harvested on April 2015 in Puglia (Italy), and transported in cold conditions to the Postharvest laboratory of the University of Foggia. The respiration activity was measured on sliced fennel after 24 h and then fennel heads were processed as described in the previous experiment. Results of the experiment 1 and previous experiment allowing to estimated the OTR for polypropylene + polyamide (PP+PA) with one layer of laser microperforation (MP1), and the quantity of fennel slices necessary to reach the target atmosphere was estimated to be 250 g. This packaging condition was compared with the same packaging filled with 150 g of samples and the conditions used in the experiment 1 PPMP1 (150 g of samples). The characteristics of each film type are reported in Table 2.7.3.2.1. All bags were sealed in passive MAP, stored at 5 °C and gas evolution into the packaging was followed for 6 days.

Table 2.7.3.2.1 Films material and packaging characteristics.

	Thickness (my)	Diameter of holes	Number of holes/m^2	OTR (ml m^{-2} day^{-1})	CO2TR (ml m^{-2} day^{-1})	β (CO2TR/OTR)
PP MP1	30	60	84	2500	2800	1.12
PP + PA MP1	67	60	110	1140	940	0.82

The evolution of gas composition within the package is showed in Figure 2.7.3.3.1. A decreased in O_2 and an increased of CO_2 levels were found in all samples. Fennel slices (150 g) stored in PP MP1 reached the steady state after 2 days, with approximately 10 kPa of both oxygen and CO_2. The use of a packaging material with less gas permeability (PP+PA MP1) allowed to reach at the equilibrium lower oxygen and higher CO_2 levels inside the bags compared to PP MP1 samples.

In particular in PP+PA MP1 bags with 150 g of fennel slices the steady state was also reached after 2 days of storage but with lower oxygen levels (7.4 ± 0.1 kPa O_2) and higher carbon dioxide concentrations (12.5 ± 0.2 kPa CO_2) compared to PP MP1 with the same fennel amount. In addition, in PP MP1 (150 g), the oxygen concentration remain almost steady until the end of the storage, while CO_2 slightly increased up to 15 ± 1.3 kPa up to the day 6. As expected in PP+PA MP1 bags with 250 g of fresh-cut fennel the desirable gas concentrations was reached. In fact by increasing the weight of the product, from 150 g to 250 g, the consumption of oxygen inside the bag was higher and, as a consequence, the O_2 level sharp decreased, reaching the desired O_2 concentration (5 kPa) already after 24 h. Then oxygen remained almost steady until the end of the storage with average values of 4.5 ± 0.5 kPa O_2. The accumulation of CO_2 was slower than O_2 depletion, and CO_2 slowly increased reaching values of 20 ± 0.9 kPa after 6 days of storage at 5 °C.

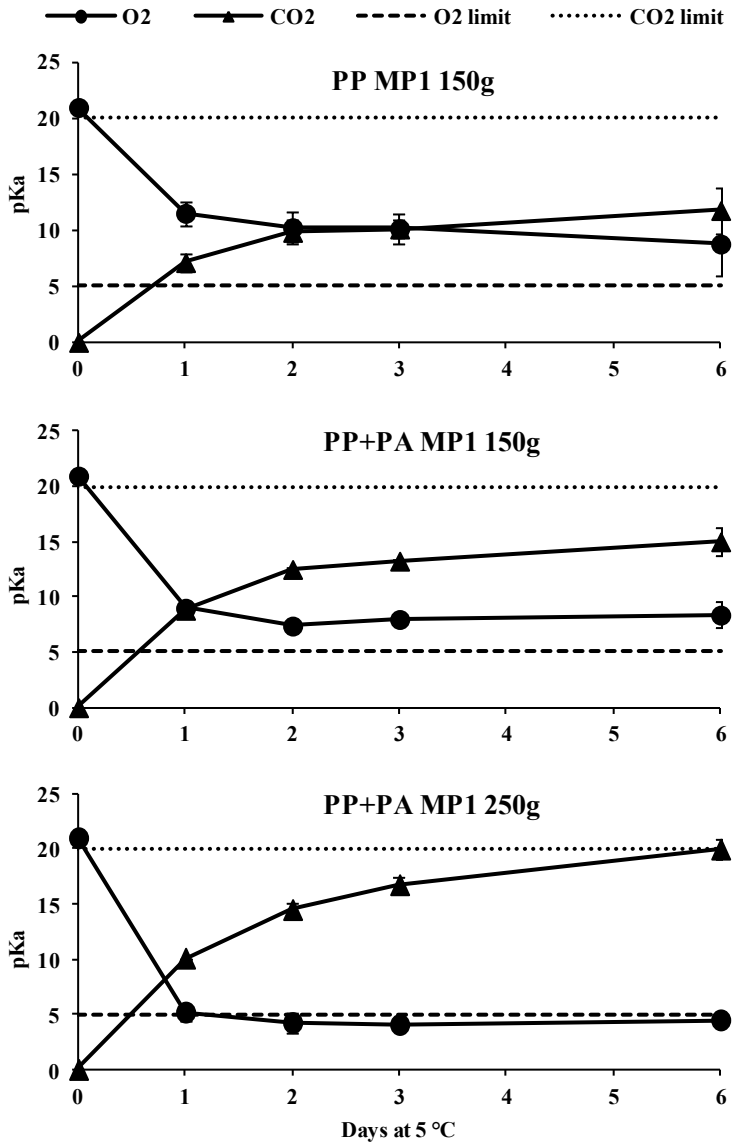

Figure 2.7.3.3.1 Oxygen and carbon dioxide concentrations within packages. Mean values of 5 replicates ± STD.

2.7.3.4 Conclusion

Results confirmed that by using PP+PA film, an higher gas barrier effect was obtained, allowing the accumulation of CO_2 above 10 kPa. In this material the increase of the product weight from 150 g to 250 g allowed to reach the target atmosphere (2-5 kPa O_2 + 20 kPa CO_2).

2.7.4 EXPERIMENT 3

2.7.4.1 Objective

Results obtained from experiments 1 and 2 allowed to optimize packaging conditions for fresh-cut fennel. The information obtained were used to perform a final experiment in which sensorial, physical chemicals and microbiological parameters on fresh-cut fennel stored in MAP were monitored during time. Therefore the objective of this experiment was to confirm the effectiveness of storage in active MAP with PP+PA MP1 film in maintaining quality of fresh-cut fennels.

2.7.4.2 Experimental setup

Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Apollo*) were harvested on May 2015 in Puglia (Italy) and transported in cold conditions to the Postharvest laboratory of the University of Foggia. As reported in previous experiments 1 and 2, the respiration activity was measured and fennel heads were

processed to obtain fresh-cut fennel samples. Values of respiration activity in cv. *Apollo* were higher (7.3 ± 0.7 mg CO_2 kg^{-1} h^{-1}) compared to that measured for cv. *Tiepolo* (5.4 ± 0.02 mg CO_2 kg^{-1} h^{-1} in EXP1 and 5.6 ± 0.2 mg CO_2 kg^{-1} h^{-1} in EXP2) therefore a lower weight of product (200 g instead of 250 g) was considered in PP+PA MP1 bags. For the packaging experiment 3 replicates of 150 g fennel slices were used for the initial determinations while the remained samples were packed as follow: 9 samples (3 replicates x 3 sampling day) of 150 g were placed in polypropylene (PP) bags with one layer of laser microperforation (MP1) and 9 samples of 200g were packed in polypropylene + polyamide (PP+PA) bags with one layer of laser microperforation (MP1). All the bags were flushed with an active modified atmosphere (5 kPa O_2 + 20 kPa CO_2 in nitrogen). The characteristics of each film type were the same reported in experiment 2 (Table 2.7.3.2.1). Additional 9 batches of 150 f of fresh-cut fennel were placed in macro-perforated polyethylene clam-packs (119 x 189 x 90 mm; capacity 500 g; CL1/90 Carton Pack®) and were used as control (CTRL). All samples were stored at 5 °C and sampling and analysis were performed after 3, 7 and 14 days. The quality attributes analyzed on treated samples in this experiment were the following:

- headspace gas composition;
- sensorial attributes (visual appearance, stem and sheath browning);
- color of stem and sheath cut-surfaces;
- weight loss;
- total soluble solid (TSS);
- pH;

- titratable acidity (TA);

- total phenols content;

- antioxidant activity;

- vitamin C (total, L-ascorbic and L-dehydroascorbic acid);

- acetaldehyde and ethanol content;

- sugars and organic acid;

- microbiological quality (mesophilic, psychrophilic, lactic acid bacteria, Enterobacteriaceae).

2.7.4.3 Results and discussion

Headspace gas composition during storage is reported in Figure 2.7.4.3.1. Starting from the initial MAP of 5 kPa O_2 + 20 kPa CO_2, in PP MP1 the steady state was reached after 3 days with 12.7 kPa O_2 and 8.7 kPa CO_2. During storage a slight increase in oxygen and a decrease in CO_2 was observed and, after 13 days at 5 °C the gas composition inside the PP MP1 bags was 15.3 kPa O_2 and 6.5 kPa CO_2. The evolution of gas composition in PP MP1 bags did not completely confirm our previous results obtained with the same packaging material, also considering the higher respiration of the product, but when using microperforated materials if the exchange through the holes is faster than the accumulation/depletion of gas, the equilibrium is very little affected by the respiration rate. In PP+PA MP1 bags, the initial gas composition was maintained over time although after 24 h of storage a decrease in O_2 (from 5 to 0.7 kPa) was observed and it was probably due to a post-cutting stress that increased the rate of respiration via ethylene (Brecht, 1995).

Then, oxygen level gradually increased, reaching the steady state at day 3 with 4.6 ± 1.1 kPa O_2. On the other hand, carbon dioxide more or less maintained initial composition throughout the experiment, although a momentary slight decrease was observed in the first 2 days of storage.

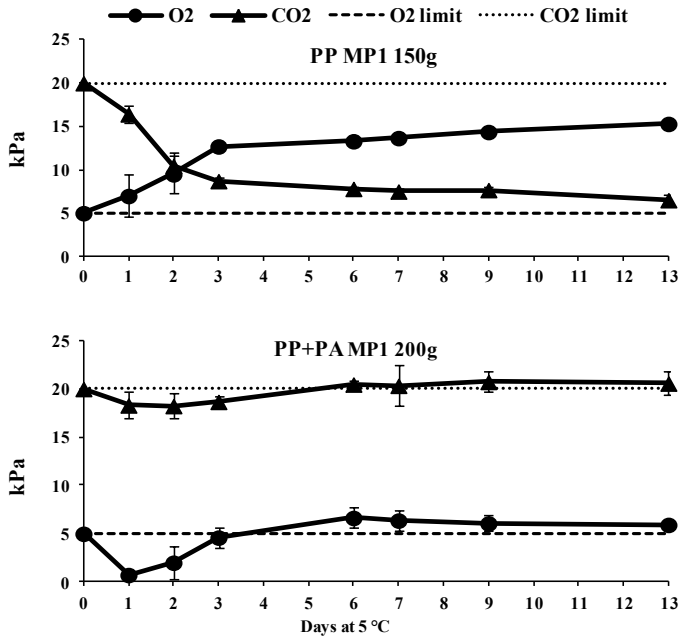

Figure 2.7.4.3.1. Oxygen and carbon dioxide concentrations within packages. Mean values of 5 replicates ± STD.

The effect of treatments, time and their interaction on sensorial, physical, chemical, and microbiological attributes of fresh-cut fennel is shown in Table 2.7.4.3.1. Different MAP conditions significantly affected sensorial attributes, the weight loss and all the color parameters of the stem part of the slices while only L*, b* and chroma were affected by treatment in the fennel sheathes. Regarding chemicals

attributes treatments significantly influenced TSS, pH, L-ascorbic acid (AA) and total vitamin C contents as well as the amount of ethanol and acetaldehyde. In addition treatments significantly affected the growth of mesophilic and lactic acid bacteria. A significant effect of time of storage was observed for sensorial attributes, weight loss and color parameters of stem cut-surface, while in regard to the sheath cut-surface storage time influenced only b* and chroma values. Among chemical attributes, titratable acidity, AA, DHA and total vitamin C significantly changed during time, as well as all the microbial counts. Interaction time x treatments resulted significant for sensorial attributes, weight loss, color parameters in stem slices, AA, vitamin C, ethanol and acetaldehyde contents, and all the microbiological parameters analyzed.

Table 2.7.4.3.1. Effect of treatments (PP MP1, PP+PA MP1 and CTRL), storage time and their interaction on quality parameters of fresh-cut fennels during storage at 5 °C. Data are mean values of 9 samples (3 replicates x 3 storage time).

Parameters	PP MP1	PP+PA MP1	CTRL	Treatment	Time	Treatment X Time
Sensorial attributes						
Visual appearance	3.8 b	4.5 a	3.1 c	****	****	**
Stem browning	1.8 b	1.2 c	2.4 a	****	****	***
Sheath browning	1.2 b	1.1 b	1.4 a	****	****	*
Physical attributes						
Weight loss (%)	0.1 b	0.1 b	0.5 a	****	****	****
Stem color						
L*	88.7 b	90.1 a	87.6 c	****	****	**
a*	-0.4 a	-1.0 b	-0.5 a	****	****	**
b*	13.2 b	11.8 c	14.8 a	****	****	***
Chroma	13.2 b	11.8 c	14.8 a	****	****	***
Hue angle	91.9 b	94.7 a	91.9 b	****	****	**
Sheath color						
L*	89.3 b	90.6 a	89.1 b	***	ns	ns
a*	-2.0	-2.0	-2.2	ns	ns	ns
b*	10.6 a	10.0 b	11.2 a	***	****	ns
Chroma	10.8 a	10.1 b	11.4 a	***	****	ns
Hue angle	100.7	101.1	101.0	ns	ns	ns
Chemical attributes						
Total soluble solid (°Brix)	6.1 b	6.2 ab	6.4 a	*	ns	ns
pH	6.4 b	6.5 a	6.3 c	****	ns	ns
Titratable acidity (mEq NaOH 100 g^{-1} fw)	1.9	1.9	2.0	ns	****	ns
Total phenol content (mg GAE 100 g^{-1} fw)	24.7	26.0	26.7	ns	ns	ns
Antioxidant activity (mg TEAC 100 g^{-1} fw)	32.3	32.9	34.9	ns	ns	ns
Ascorbic acid (mg 100 g^{-1} fw)	4.9 b	5.6 ab	6.5 a	**	****	****
L-dehydroascorbic acid (mg 100 g^{-1} fw)	2.7	2.9	3.1	ns	***	ns
Vitamin C (mg 100 g^{-1} fw)	7.6 b	8.5 ab	9.6 a	**	****	***
Ethanol (ul L^{-1})	7.9 b	57.8 a	4.8 b	****	ns	****
Acetaldehyde (ul L^{-1})	1.1 b	2.6 a	0.7 b	****	ns	**
Sugars (g 100g^{-1} fw)						
Fructose	1.9	1.7	2.3	ns	ns	ns
Glucose	1.5	1.2	1.8	ns	ns	ns
Sucrose	0.8	0.6	0.3	ns	ns	ns
Organic acid (mg 100g-1 fw)						
Oxalic acid	12.5	9.8	16.3	ns	ns	ns
Citric acid	20.2	21.8	12.1	ns	ns	ns
Tartaric acid	11.7	9.1	13.8	ns	ns	ns
Malic acid	577.6	470.9	579.5	ns	ns	ns
Quinic acid	28.5	24.9	24.1	ns	ns	ns
Succinic acid	243.3	251.3	280.1	ns	ns	ns
Fumaric acid	14.5	18.7	12.1	ns	ns	ns
Microbiologica quality						
Mesophilic bacteria (log CFU g^{-1})	5.4 b	5.3 b	6.2 a	****	****	***
Psychrophilic bacteria (log CFU g^{-1})	5.5	5.3	5.7	ns	****	**
Lactic acid bacteria (log CFU g^{-1})	2.8 a	2.4 b	2.0 c	****	****	****
Enterobacteriaceae (log CFU g^{-1})	3.2	2.9	3.2	ns	****	***

Asterisks indicate the significance level for each factor of the ANOVA test (ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$). Different

letters indicate statistical differences within treatments, according to the Tukey's test ($p \leq 0.05$).

To better understand the interactive effect of time and treatments on sensorial attributes of fresh-cut fennels, in Figure 2.7.4.3.2 and are shown changes over time of visual appearance and browning score for stem and sheath cut-surfaces. Significant differences in visual appearance were observed at each sampling date between fresh-cut fennels stored in PP+PA MP1 compared to CTRL. The appearance score of fennel slices in PP+PA MP1 bags remained unchanged to values of 4 throughout storage time while CTRL gradually lost visual appearance but it remained still marketable up to the last sampling day (day 13). In PP MP1 the appearance score decreased more slowly than the CTRL, reaching a value of 3.5 at day 7, significantly lower compared to PP+PA MP1 and higher compared to CTRL, whereas after 14 days it was not different from CTRL samples. Fresh-cut fennel stored in air (CTRL) showed an increase of browning of the stem during time up to a score of 2.6 after 7 day and then values did not change up to 13 days at 5 °C. According to the visual appearance evaluation, after 3 days no differences were observed in the browning of the stem among treatments in MAP. A very slight presence of browning started to be observed after 7 days in PP MP1 and after 13 days values of stem browning were similar in PP MP1 and CTRL, while no development of browning was observed over time in fresh-cut fennel stored in PP+PA MP1 for both stem and sheath. In general the score of browning in the sheath part was lower than 2 in all treatments despite significant differences were found between CTRL and PP+PA MP1 after 7 and 13 days. In PP MP1 the

browning on the sheath part occurred in the last 6 days of storage when fennel sheathes were scored 1.4.

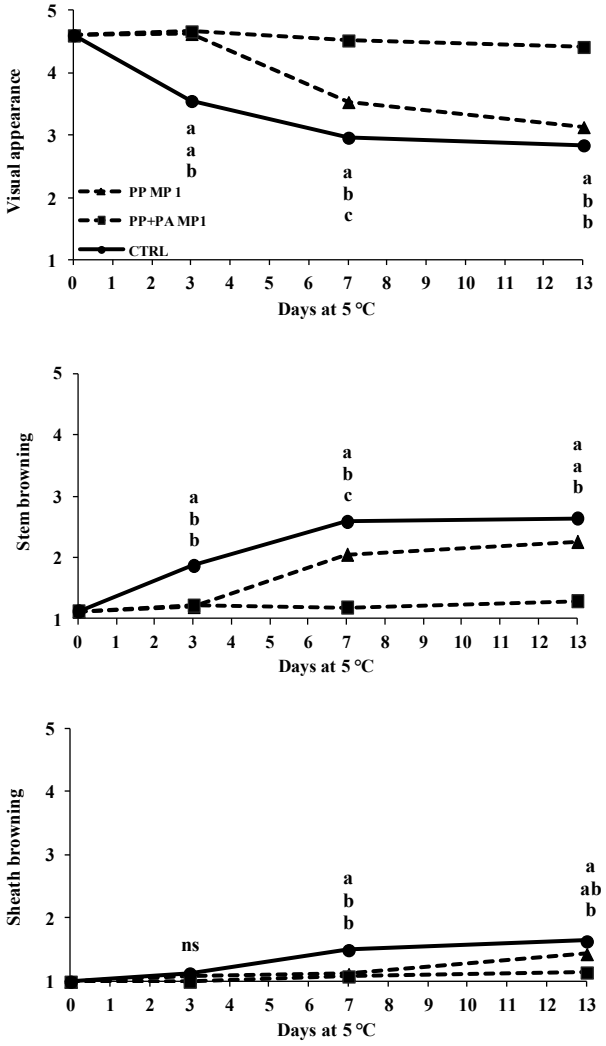

Figure 2.7.4.3.2 Sensorial attributes (visual appearance, stem and sheath browning) scores of fresh-cut fennel during storage at 5 °C. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Visual appearance scale: 5 = excellent, 4 = good, 3 = fair, 2 = poor, 1 = very poor. Browning scale: 1= absence of browning, 3= slight browning, 5= completely brown.

The beneficial effects of MAP on visual appearance as well as the stem browning evaluation on fennel slices were already reported in experiment 1 and sensorial data in the present study confirmed that results. Comparing MAP samples, the higher visual appearance and lower stem browning scores were observed in fresh-cut fennels stored in PP+PA MP1 where the optimum gas concentrations were reached. Therefore in the present study the main factor that affected the appearance of fresh-cut fennel was the gas composition inside the bags. The importance of atmosphere composition that surrounds the commodity during storage was extensively reported by many authors (Zagory et al., 1988; Gorny, 1997; Kader, 2002a; Kader, 2002c; Hoehn et al., 2009). The better visual appearance of fennel slices observed in PP+PA MP1 bags compared to PP MP1 in the present study confirmed the effectiveness of gas composition selected in previous experiment with CA (Experiment 2.6) on visual quality of fresh-cut fennel during storage at 5 °C. In addition, taking also into account the results of sensorial evaluations, it was confirmed that even when fennel slices were dipped in ethanol the most effective gas composition to preserve quality was confirmed to be 5 kPa O_2 and 20 kPa CO_2 as reported by Escalona et al. (2005b). In the present experiment in fact, despite the storage temperature was higher (5 °C versus 0 °C), fresh-cut fennel in PP+PA MP1 bags did not show significant changes in visual appearance as well as in browning in both stem and sheath, even after 13 days. Results from sensorial analysis are

supported by color data as evaluated on the stem cut-surface (Table 2.7.4.3.1). Fresh-cut fennels stored in PP+PA MP1 had highest L* and hue angle values, as well as lowest a*, b* and chroma values. In addition PP MP1 samples had higher L*, b* and chroma values compared to CTRL, while a* and hue angle values were similar. Changes during storage in color parameters assessed on the stem cut-surfaces are shown in Figure 2.7.4.3.3. L* value remained unchanged in PP+PA MP1 over time while in CTRL samples the lightness decreased during storage, being significantly lower than PP+PA MP1 samples at each storage time. A decrease in L* values was also observed in PP MP1 samples although it started later than for air and became significantly lower that PP+PA MP1 only after 7 and 13 days of storage. Regardless of the treatment, after 3 days at 5 °C, stem a* values increased but remained in the negative part of the axis. No further changes in a* values were observed during storage in PP+PA MP1 samples while in PP MP1 and CTRL samples it continued to increase, being significantly higher than PP+PA MP1 at each sampling date. No changes in b* values were observed in PP+PA MP1 samples during storage, while in CTRL samples this color parameter increased over time, with values significantly higher than PP+PA MP1 at each sampling date and similar to PP MP1 at the end of the storage. Chromaticity changes over time are not shown: they reflect the same pattern of b* values. Hue angle of the stem slight decreased after 3 day in all samples, with a minor incidence in PP+PA MP1 samples compared to other treatments at days 3 and until the end of the storage. Color data of the stem cut-surface confirmed that the browning was inhibited in

samples stored in PP+PA MP1 while the highest color changes were observed in samples stored in air.

In fresh-cut fennel stored in PP MP1 was observed a delay in the occurrence of browning in the stem cut-surfaces; in particular in the first three days of storage, when the oxygen level increased from 5 to 12.7 kPa, color parameters were similar to that in PP+PA MP1. Browning started to occur in PP MP1 samples from the third day of storage, and after 14 days at 5 °C stem color in PP MP1 and CTRL had similar values, in accordance with results of the browning score of the stem judged by panelists; the inability of PP MP1 film to delay browning was probably due to the relative high O_2 level reached during storage. Likewise the inhibition of the browning development in fresh-cut fennel stored in PP+PA MP1 was related to the low oxygen levels that were maintained during storage.

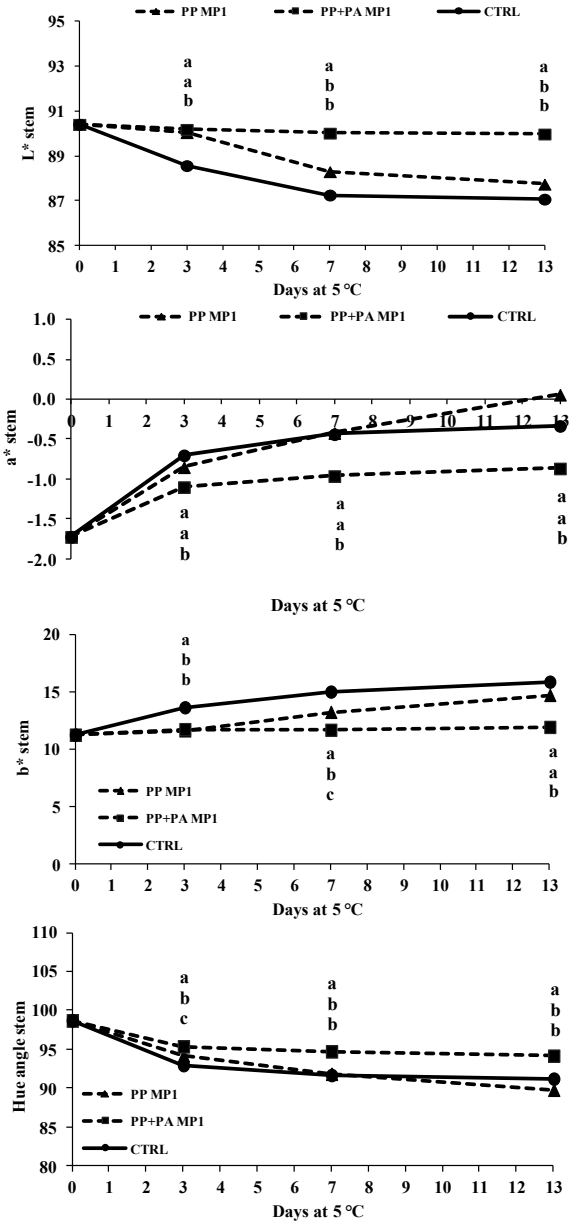

Figure 2.7.4.3.3 Changes in color parameters on stem part of fresh-cut fennel during storage at 5 C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

No significant interactions time x treatments were found in regard to color parameters in the sheath cut-surface (Table 2.7.4.3.1). PP+PA MP1 samples showed highest L* and lowest b* and chroma values, while no differences were observed between PP MP1 and CTRL samples. In addition the a* and hue angle values did not show any significant change during time and in relation to treatments. According to our previous results in CA and also in MAP, browning of the stem parts of the fennel slices was the aspect that more affected the visual appearance and, in turn, the marketability.

Changes in samples weight loss during storage is showed in Figure 2.7.4.3.4. A significant loss of weight over time was observed in all treatments. As expected the highest weight loss occurred in fresh-cut fennel stored in air (CTRL) with significant differences compared to MAP samples at each sampling date. These differences were due to the presence, in CTRL samples, of macro-perforations that allowed higher gas exchanges, but also an increase in dehydration, due to water transpiration and, consequently, weight loss.

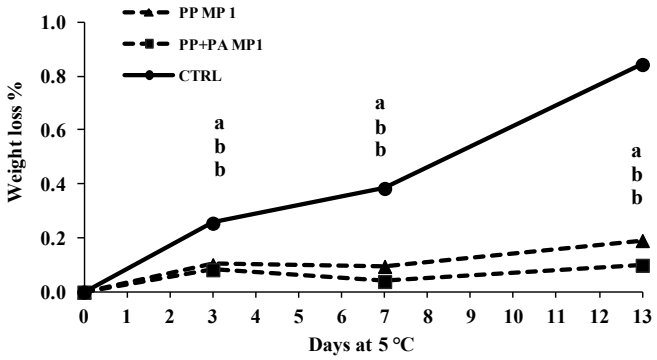

Figure 2.7.4.3.4 Weight loss in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

A slight effect of treatment ($p \leq 0.05$) was found for TSS: values ranged from 6.0 to 6.6 with CTRL samples presenting the highest content of TSS, probably due to the weight loss that caused solute concentrations. Sugars are generally considered to be the main contributors to TSS in fresh fruits and vegetables, together with organic acids, vitamins and minerals (Zhan et al., 2014). Nonetheless, in our experiment HPLC analysis of sugars and organic acid profiles did not show any significant differences between treatments and during storage (Table 2.7.4.3.1). In addition no significant changes in titratable acidity were observed during storage and in regard of treatments (Table 2.7.4.3.1). Among sugars, fructose and glucose were respectively the first and second most abundant sugars in fennel slices (1.9 ± 0.9 g 100 g^{-1} fw and 1.5 ± 0.7 g 100 g^{-1} fw). Cataldi et al. (1998) reported D-glucose and D-fructose as the main sugars in fennels. Similar results on fennel cv. Orion were

reported by Escalona et al. (2004, 2005a and 2006) despite in their experiment the amount of glucose was higher compared to fructose. Also Barros et al. (2010), that measures macronutrients profiles of fennels, found that glucose was the most abundant sugar. Differences in ration glucose/fructose could be probably related to the different cultivar analyzed. Variability in sugar composition among different genotypes or cultivars were previously observed in seabuckthorn berries (Raffo et al., 2004), carrots (Suojala, 2000) and apple juice (Karadeniz et al., 2002). Among organic acid analyzed, malic acid was the most abundant (537.6 ± 227.6 mg 100 g^{-1} fw), followed by succinic (263.1 ± 130.5 mg 100 g^{-1} fw), quinic (26.2 ± 15.4 mg 100 g^{-1} fw), citric (17.05 ± 12.9 mg 100 g^{-1} fw), fumaric (15.1 ± 8.6 mg 100 g^{-1} fw), oxalic (12.9 ± 5.6 mg 100 g^{-1} fw) and tartaric acid (11.6 ± 5.4 mg 100 g^{-1} fw). Pereira et al., (2013) found that the main organic acid in fennel flowers of *Foeniculum vulgare* was malic acid while Escalona et al. (2006) reported that oxalic acid is the main organic acid of fennel bulbs with the amount of 30.4 mg per 100 ml juice at harvest. In wild fennels Sánchez-Mata et al., 2012 reported an average value of oxalic acid of about 250 mg per 100 g of homogenate, and it was also the most abundant among organic acid analyzed. However it must be considered that the quantification of oxalic acid depends on the type of extraction method since the total oxalate is extracted in strong-acid solution while water can extract only the water soluble part (Libert et al., 1987). Thus in the present study where water was used for the extraction, the oxalic acid detected was related to the water soluble fraction, in contrast to Sánchez-Mata et al. (2012) that used phosphoric acid, obtaining both soluble and insoluble water fractions. Regarding data reported by

Escalona et al. (2006) instead the analysis was carried out directly on juice samples, however the authors did not report the total organic acid profile therefore a comparison with our data is not appropriate.

As showed in Figure 2.7.4.3.5, in CTRL samples vitamin C and AA contents remained almost stable while a significant decrease was observed at the end of the storage in fresh-cut fennels stored in MAP, without differences between PP MP1 and PP+PA MP1 samples. Comparing AA in CTRL and MAP samples, our results are in agreement with many studies on other crops which report a decrease in AA content when they are stored with high CO_2 compared to samples held in air or with low oxygen as for potato strips (Tudela et al., 2002), fresh-cut kiwifruit slices (Agar et al., 1999),'Conference' pear (Veltman et al., 1999), and rocket leaves (Martínez-Sánchez et al., 2006). It was previously reported that in fresh-cut products high CO_2 concentrations in the storage atmosphere cause degradation of vitamin C because carbon dioxide may stimulate the oxidation of ascorbic acid by increasing ascorbate peroxidase activity (Mehlhorn, 1990; Devlieghere et al., 2002). In accordance with these authors, the decrease in vitamin C in both PP+PA MP1 and PP MP1 samples could be due to the presence of higher CO_2 concentrations in MAP compared to that in fresh-cut fennel held in air.

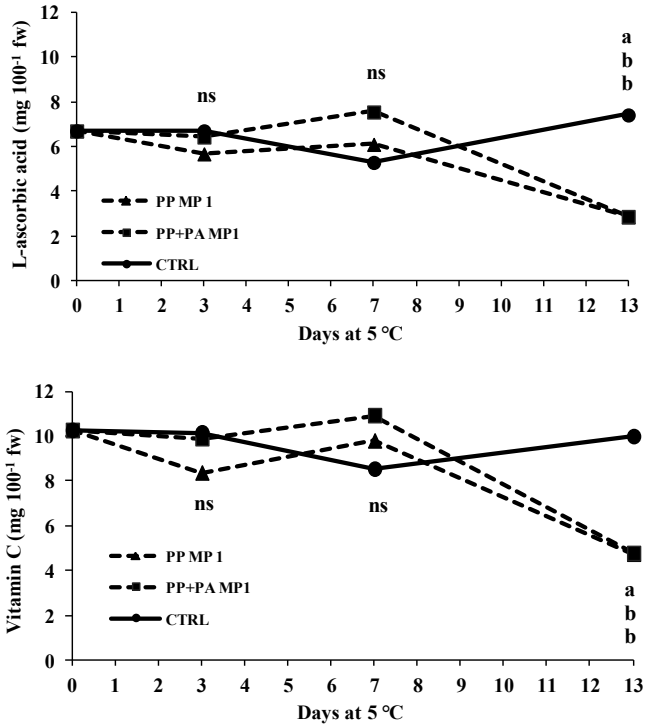

Figure 2.7.4.3.5 Changes in total vitamin C and L-ascorbic acid contents in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

Samples stored in PP+PA MP1 showed highest values of both contents of ethanol and acetaldehyde while samples PP MP1 and CTRL had similar contents (Table 2.7.4.3.1).

Changes in ethanol and acetaldehyde during storage are reported in Figure 2.7.4.3.6. Initial content of ethanol and acetaldehyde were 13.2 ± 2.11 μl L^{-1} and 0.84 ± 0.01 μl L^{-1} respectively. Experiments conducted by Kimmerer et al. (1982)

showed that ethanol and acetaldehyde production by plants does not require restricted O_2 availability, but it was related to a substantial alteration of respiratory metabolism in stressed plants. Considering that wounding is one of the primary stresses experienced by fresh-cut produce (Hodges et al., 2008), the initial content of both ethanol and acetaldehyde before packaging could be associated to a wounding response. Also Smyth et al. (1999) detected ethanol in shredded carrots immediately after packaging and made the hypothesis that fermentation occurring before or immediately after cutting, was perhaps a wounding response. In addition, in the case of ethanol the initial content was probably partly due to the use of this alcohol during processing operations as anti-browning agent. Regards treatments, fennel slices in PP+PA MP1 had a significant increase in ethanol and acetaldehyde contents during storage and values were significantly higher compared to PP MP1 and CTRL samples at each sampling day. After 14 days ethanol and acetaldehyde in PP+PA MP1 reached values of 66.1 ± 2.8 and 2.9 ± 0.3 μl L^{-1} fw respectively. These increase were probably caused by to low level of oxygen (0.73 kPa) reached after 24 h since oxygen concentrations below 1-2 kPa can lead to anaerobic metabolism, and is associated to the production of ethanol and acetaldehyde. When MAP atmosphere becomes anaerobic in fact, the primary response of fresh fruits and vegetables is to produce elevated concentrations of ethanol and to a lesser extent acetaldehyde, resulting in off-flavors, off-odors, and loss of quality (Brandenburg et al., 2009; Forney et al., 2009). In addition, as previously observed in the first packaging experiment and in that of CA reported in this dissertation (Experiment 2.6), also high CO_2 can lead to an increase in fermentative volatiles,

mainly ethanol. However, the ethanol concentration reached in PP+PA MP1 samples should not be high enough to perceive off-odors since the odor threshold for ethanol is 100 μl L^{-1} as reported by Flath et al. (1967).

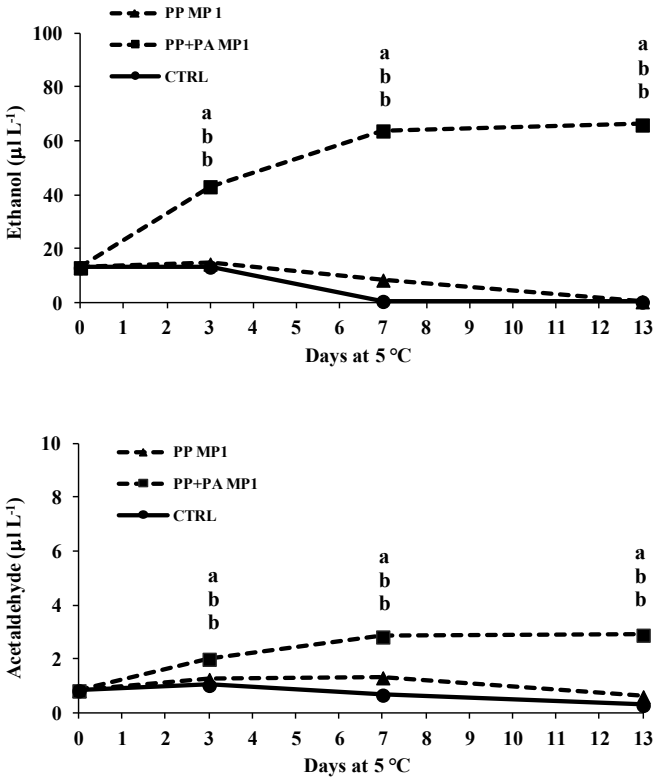

Figure 2.7.4.3.6 Changes in ethanol and acetaldehyde contents in fresh-cut fennel during storage at 5 C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

The microbiological quality assessed on fresh-cut fennels was significantly affected by interaction time x treatment (Table 2.7.4.3.1). The effect of treatment on mesophilic, psychrophilic, lactic acid bacteria, ad Enterobacteriaceae at each sampling day is showed in Figure 2.7.4.3.7.

The initial counts of mesophilic and psychrophilic bacteria were 4.08 ± 0.35 and 3.2 ± 0.2 log CFU g^{-1}, respectively. Comparing these data with results obtained on fresh-cut fennels by Escalona et al. (2006), the initial microbial contamination in the present study, in terms of mesophilic and psychrophilic bacteria was lower: the authors reported an initial mesophilic count of 5.2 ± 0.3 log CFU g^{-1} and similar amounts of psychrophilic bacteria assessed after two disinfectant washes, before (50 mg L^{-1} NaOCl solution) and after (100 mg L^{-1} NaOCl solution) cutting. A higher psychrophilic bacteria count after processing (from 4.0 to 4.97 log CFU g^{-1}) compared to data in this experiment was also reported on fresh-cut fennels by Albenzio et al. (1998). In the preset experiment the disinfection was assessed only on whole fennels, before cutting, therefore the lower mesophilic and psychrophilic bacteria compared to data reported by Escalona et al. (2006) and Albenzio et al. (1988) suggest a better microbiological quality of the fresh product at harvest. In addition no yeasts and moulds were detected at time zero and during storage while the initial levels of lactic acid bacteria and enterobacteriaceae were less than 2 and 1 log CFU g^{-1} respectively. Similarly Albenzio et al. (1998) reported very low level of lactic acid bacteria (1 log CFU g^{-1}) and enterobacteriaceae (from 1.48 to 1.60 log CFU g^{-1}) after processing while the yeasts counts were below 1 log CFU g^{-1} during storage.

Regardless of the treatment, all bacterial populations increased over time although the amounts after 14 days at 5 °C were below the limit prescribed by French standard limits for microbial counts (7.7 log CFU g^{-1}) for the marketability of fresh-cut products. (DGCCRF 1993). The growth of mesophilic bacteria was delayed in MAP samples: fresh-cut fennel stored in PP MP1 and PP+PA MP1 had significant lower count of mesophilic bacteria compared to CTRL up to 7 days of storage, when the use of modified atmosphere allowed to reduce the growth of 1 log. After 13 days no significant differences were observed in all treatments and the mesophilic counts were about 6.5 log CFU g^{-1}. Similar results were reported on celery sticks (Gómez et al., 2005) and in fresh-cut mushrooms (Capotorto et al., 2015) in which the mesophilic bacteria growth was lower for MAP than for control in air. Also in the case of Enterobacteriaceae, CTRL had significant higher count (4.26 ± 0.21 log CFU g^{-1}) compared to MAP samples (3.40 ± 0.27 log CFU g^{-1} in PP MP1 and 3.20 ± 0.30 log CFU g^{-1} in PP+PA MP1) after 7 days of storage, while there were no significant differences among treatments at last sampling day where the Enterobacteriaceae counts were about 4.75 log CFU g^{-1}. The effect of MAP on the inhibition of both mesophilic bacteria and Enterobacteriaceae could be due to the presence of high CO_2 inside the bags compared to samples in air. Concentrations of CO_2 at 5 to 10% are usually needed to have an effect on microbial growth (Cantwell et al., 2002). Zagory (1999) reported that elevated CO_2 extends the lag phase of bacterial growth and can slow the propagation of bacteria. As a consequence, the growth was not inhibited but only delayed, in fact after 14 days the counts of mesophilic bacteria and Enterobacteriaceae in fresh-cut fennel

stored in MAP were similar to CTRL samples. Concerning psychrophilic bacteria, the effect of MAP was less evident: despite CTRL samples had the higher count after 3 (4.90 ± 0.34 log CFU g^{-1}) and 7 (5.97 ± 0.35 log CFU g^{-1}) days of storage, significant differences were detected at day 3 compared to fennel slices stored in PP+PA MP1 (3.95 ± 0.22 log CFU g^{-1}) while after 7 days the lower count of psychrophilic bacteria was in PP MP1(5.04 ± 0.33 log CFU g^{-1}). At the end of the storage psychrophilic bacteria counts were similar in all samples, and the mean value of about 6.44. Similarly Escalona et al. (2006) did not found significant differences in psychrophilic counts during storage in fennel slices stored at 5 °C under different controlled atmospheres. During storage and up to 7 days lactic acid bacteria counts remained below 2 log CFU g^{-1} and it could depends on the metabolism of these bacteria; in fact it has been previously reported that, because of the low energy yields, lactic acid bacteria often grow more slowly than microbes capable of respiration (Barth et al., 2009). At last sampling day the counts of lactic acid bacteria increased only in MAP samples, reaching values of 4.66 ± 0.27 log CFU g^{-1} in PP MP1 and 3.48 ± 0.22 log CFU g^{-1} in PP+PA MP1, while in CTRL the count was 2.11 ± 0.4 log CFU g^{-1}. Farber et al. (2003) reported that the effect of MAP on lactic acid bacteria can vary depending on the type of product: the increased CO_2 and decreased O_2 concentrations used in MAP generally favor the growth of lactic acid bacteria. On this regards, results in the present study are partially in agree with Faber et al. (2003): lactic acid bacteria were significantly higher in MAP samples compared to CTRL; on the other hand, comparing PP MP1 and PP+PA MP1, the highest count was assessed in PP MP1 that has a lower CO_2

and higher O_2 concentrations compared to PP+PA MP1. The lactic acid bacteria can only obtain ATP by fermentation, usually of sugars, and the fermentation lowers the pH due to the lactic acid production (Barth et al., 2009). Therefore it is possible that the significantly differences in lactic acid bacteria amount in MAP can explain differences in pH and TSS in these samples. Comparing MAP samples in fact, fresh-cut fennel stored in PP MP1 had significantly lower pH as well as lower TSS content compared to samples in PP+PA MP1, probably because of the highest lactic acid bacteria amount in PP MP1 that used sugars, lowering the pH.

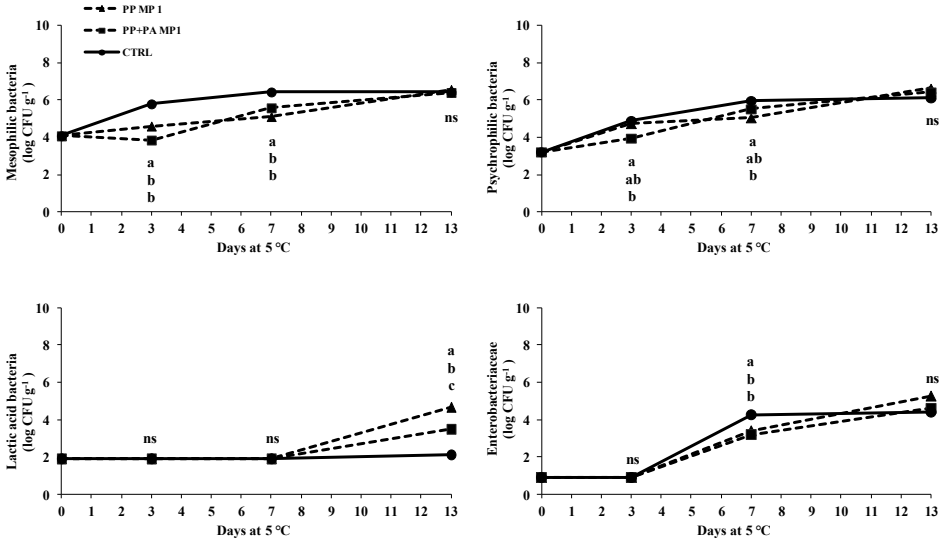

Figure 2.7.4.3.7 Changes in microbial growth in fresh-cut fennel during storage at 5 °C. Values are mean of three replicates for each treatment. Within the same storage evaluation, different letters indicate statistical differences, according to the Tukey's test ($p \leq 0.05$).

MASLT Approach

For the estimation of the shelf-life with a multivariate approach only the most important parameters for which the limit of marketability is known were included, and particularly weight loss, appearance score (VQ), browning of the stems, browning of the sheathes, Vitamin C, ethanol, acetaldehyde, mesophilic, psychrophilic, lactic bacteria and Enterobacteriaceae. Imposed limit were score 3 for the sensorial scores, 0.5% for weight loss, 5 mg/100 g for Vitamin C, corresponding to 1/8 of the daily recommended intake, according to the Australia and New Zealand Food Authority (ANZFA, 2002), 100 µL/L for ethanol, 0.015 µL/L for acetaldehyde (Flath et al., 1967), 7 CFU for mesophilic (DGCCRF 1993) and psychrophilic (AFSCA, 2012; Utyttendaele et al., 2010), 6 for lactic bacteria (FCD, 2009), and 3 for Enterobacteriaceae (DGCCRF 1993).

The PCA conducted on these data showed that the model accounted for 79.43% of the total variance of experimental data analyzed, with PC1 and PC2 explaining 51.33% and 28.10% of the variance, respectively. The loading of the attributes analyzed on the PC1-PC2 plane is showed in Figure 2.7.4.3.8.

Most of the variable had the highest load in the PC1, while Vitamin C, lactic bacteria, ethanol and acetaldehyde contents had a weight on PC2, comparable to that of browning and visual quality score, and the remaining groups of bacteria, on PC1. Particularly visual quality score, ethanol and acetaldehyde were in the negative part of both PC1 and PC2; Vitamin C was in the negative part of PC1 and positive axes for PC2, whereas weight loss and browning scores (stem and sheath)

were positively correlated to PC1 and PC2; finally in the lower right quadrant with positive PC1 and negative PC2 were all the microbial groups.

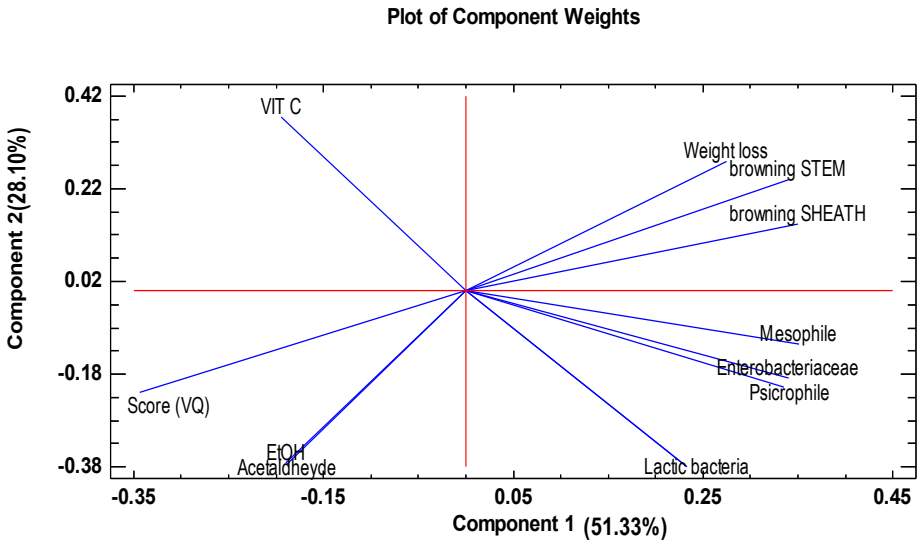

Figure 2.7.4.3.8 Loading factors describing the relationship among quality attributes of fresh-cut fennel stored for 13 days at 5 °C under different conditions.

Initial samples were characterized by high visual quality score and Vitamin C, and by low stem and sheath browning scores as well as low microbial counts (Fig. 3.8 and 3.9) With the increase of storage time samples moved to the right part of the graph, due to the increase of browning score, weight loss and microbial load while samples packaged in PP+PA MP1, showing less variation in term of PC1 scores, and more variation on PC2, moved to the negative part due to the increase of ethanol and acetaldehyde.

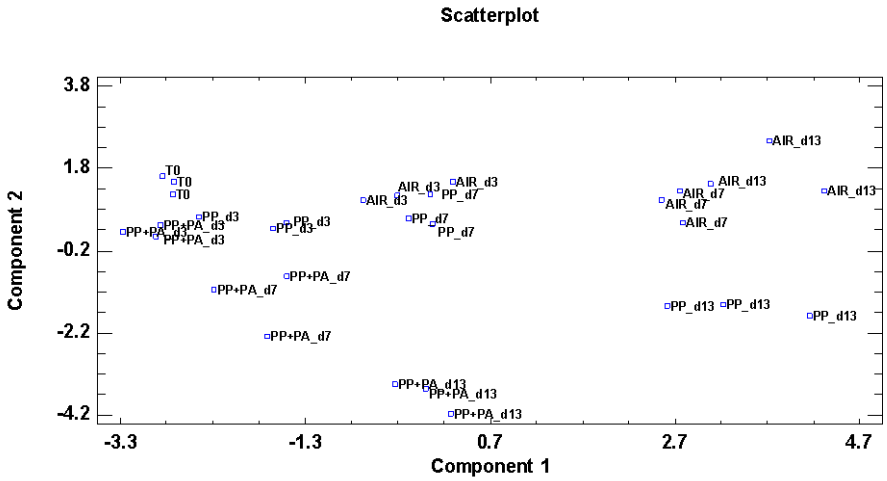

Figure 2.7.4.3.9 Score plot describing the relationship among treatments at each sampling day for fresh-cut fennels stored 13 days at 5 °C under different storage conditions.

Scores of PC1 for each storage treatment showed a strong relation with time, as shown in Figure 2.7.4.3.10. This relation was clearly linear for the two MAP conditions, while it seemed more as an exponential curve for samples stored in AIR (even if starting from negative values). Standing to these consideration the curves of the score changes for PP+PA MP1 and PP MP1 were fit by a zero order reaction, whereas different equations were tested for AIR samples. Results of the best fitting for each curve are reported in Table 2.7.4.3.2. For PP MP1 and PP+PA MP1, the zero order kinetic allowed to explain 95% and 92% of the total variance respectively. The kinetic rate for PP MP1 was almost double than for PP+PA MP1, being 49% and 26% respectively. As for the fitting in AIR the exponential equation is shown in Table 2.7.4.3.2; also in this case an intercept was estimated, being 4.68,

whereas the exponential was multiplied by a coefficient corresponding to -7.68. Being the curve not of the same order, a direct comparison of the kinetic rate cannot be made, but the MALST analysis allowed to estimate the shelf-life for each condition. A cut-off limit was, in fact, obtained by inserting, for each quality parameter, its critical value for the product marketability, as indicted by Amodio et al. (2015). This limit is calculated by choosing the highest value of the multiplication product of auto-scaled values of the reference limits of each quality attribute and its respective loading. In this experiment this limit was due to the sheath browning and resulted in a cutoff value of 2.8. The intersection of this value with the score curves allowed to estimate the shelf-life for each storage conditions, being 9.7, 12.2 and 24.2 days in AIR, PP MP1 and PP+PA MP1 respectively.

Table 2.7.4.3.2 Kinetic parameters of the best fitting obtained for PC1 scores as a function of time for each storage conditions.

			Rate constant	
	Equation	**R^2**	**Lower limit**	**Upper limit**
AIR	y = -7.68*exp(-(0.17)*time)+4.68	0.94	0.067	0.27
PP MP1	y = -3.25+0.49*time	0.95	0.41	0.58
PP+PA MP1	y = -3.56+0.26*time	0.92	0.19	0.33

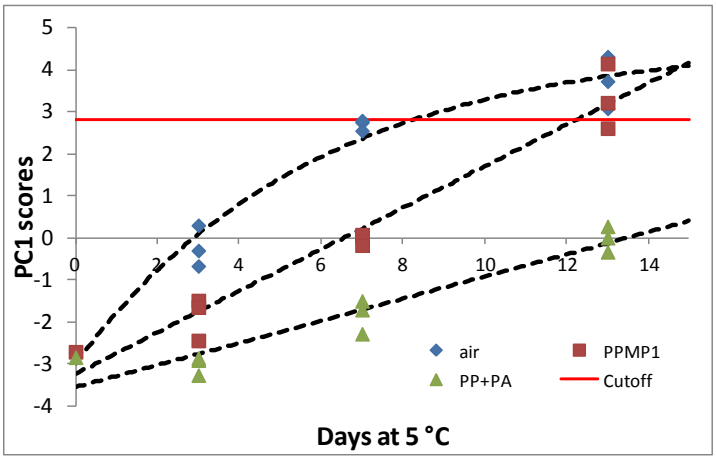

Figure 2.7.4.3.10 Curve fitting obtained for the PC1 scores as a function of time for each storage conditions. The intersection of the cut-off line with each curve defines the shelf-life for each treatment.

Escalona et al. (2005a) reported that fresh-cut fennels stored at 0 °C, in different packaging materials, with about 10% CO_2 and O_2 were still acceptable for commercial purpose after 14 days, but these authors did not apply any method to estimate shelf-life. Moreover there are very few studies using this approach for fresh-cut products. Multivariate approach compared to the conventional approach, is considering the overall degradation of the products, including several parameters which are considered critical for shelf-life estimation, allowing a more accurate prediction. The conventional method to calculate shelf-life is in fact defined on the basis of one quality parameter, which is normally defined by regulations (i.e., safety limits), or delegated by stakeholders (i.e., scientists, processors, and consumers) and that is not always the most critical for the shelf-life. The result of the shelf-life estimation, therefore, when the quality of food is limited by another or by multiple

attributes, may produce significant discrepancies. For fresh-cut products, in fact, quality is rapidly affected by the contemporaneous changes of several attributes during storage and given to the fact that the shelf-life is usually limited to 1 or 2 weeks, an accurate estimation, although difficult to obtain, is of paramount importance.

2.7.4.4 Conclusion

The PP+PA MP1 bags optimized in this last experiment for the fennel respiration rate, allowed to reach the target gas composition (5 kPa O_2 + 20 kPa CO_2) which was maintained during 13 days of storage at 5 °C. Therefore, based on results of the present experiments, packaging 200-250g of fennel slices (depending on the respiration rate) in PP+PA MP1 bags (15 x 20 cm), after a dipping in ethanol (0.5%), with initial gas composition of 5 kPa O_2 and 20 kPa CO_2 was effective in preserving visual and nutritional quality. In addition, by applying the Multivariate Accelerated Shelf Life Testing (MASLT) the shelf-life of fresh-cut fennels stored in this condition was estimated to be 24 days at 5 °C, which is much higher compared to those found in other studies, allowing a better management of the logistic chain for this product.

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PART THREE:CONCLUSIONS

3.1 GENERAL CONCLUSIONS

This thesis work was aimed to study some of the critical aspects influencing suitability of fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum*) to minimally processing, in order to optimize the technology and increase its potential marketability as a fresh-cut product.

To achieve this purpose, a number of experimental trials were carried out, with the following objectives

- evaluate the effect of maturity at harvest on quality characteristic and chemical composition of fennels and their browning susceptibility when processed as a fresh-cut products;
- detect the capability of Vis/NIR spectroscopy for the prediction of quality attributes of fennels at different harvest times;
- investigate the effectiveness of different antibrowning solutions on maintaining quality characteristics of fresh-cut fennel during storage;
- identify best suitable gas mixture to extend the shelf-life of fresh-cut fennel;
- design a modified atmosphere packaging to extend the shelf-life of fresh-cut fennel.

On the base of the results obtained, it can be concluded that:

- the commercial maturity stage ensures the full size of the commodity, with high nutritional values and good sugars contents;
- to process fennel heads as fresh-cut product, a slight anticipation of harvest time, in relation to the commercial maturity stage, could reduce the occurrence of browning of fennel slices during post-cutting storage;

- the calibration models in the visible range (Vis/NIR spectral range) presented better results as compared to those in the NIR;
- hyperspectral imaging in the VIS-NIR spectral range can be used to predict internal content of soluble solids, phenols and antioxidant activity and to classify fennel heads according to the harvest time;
- dipping in 0.5% ethanol for 2 min could represent a useful pretreatment for extending the shelf-life of fresh-cut fennel;
- the presence of 20 kPa CO_2 in the gas mixture may delay browning of fresh-cut fennel, while low O_2 concentrations (2 – 5 kPa) in the CO_2 enriched atmosphere only slightly affected the visual quality;
- a CA of 20 kPa CO_2 in air was useful to preserve visual appearance of fresh-cut fennels stored at 5 °C for 14 days, delaying the occurrence of browning on the cut surfaces; these results were also confirmed by the multivariate analysis;
- the use of microperforated PP+PA film bags with an initial gas concentration of 5 O_2 kPa and 20 CO_2 kPa maintained the quality of fresh-cut fennel for 13 day at 5 °C;
- in this MAP condition, using Multivariate Accelerated Shelf Life Testing (MALST) , a shelf-life of 24 days was predicted.

The results of this thesis increased the knowledge on some of the critical aspects for minimally processing fennel, providing important information to improve pre- and post-cutting handling in order to remove technological constrains for the production of a value-added, convenient, ready-to-eat fennel product.

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