Postharvest Characterization of Kale (*Brassica oleracea* L. var *acephala* DC.): A Study of the Impact of Leaf Maturity, Fresh-Cut Preparation and Storage Conditions

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**Abstract**

Kale (*Brassica oleracea* L. var *acephala* DC.) is a leafy vegetable that has experienced a dramatic increase in consumption in the U.S. market recently due to its nutritional content and health-associate benefits. Currently it is consumed as intact leaves as well as fresh-cut processed. Kale is harvested several times from the same plant and leaves may be harvested at different stages of maturity. To determine the potential impact of leaf maturity in combination with fresh-cut processing and storage temperature on the postharvest performance of kale, intact or fresh-cut leaves at different stages of maturity were evaluated at a range of temperatures (0 to 20°C) in air or modified atmospheres. Kale leaves were obtained from two cultivars (‘Winterbor’ and ‘Lacinato’) and different commercial sources. Product was evaluated for marketable quality attributes (visual quality, yellowing, decay, discoloration, off-odors), for color by destructive and non-destructive techniques (pigment extraction, reflectance colorimeter, reflectance spectrophotometer, SPAD), respiration, and chemical composition (ammonia, malondialdehyde, antioxidants and pigment extraction). Immature leaves had the highest antioxidant capacity and the lowest contents of ammonia and malondialdehyde over time, as well as the highest marketable quality scores. Overmature leaves had the highest rates of deterioration, the highest total ammonia and malondialdehyde contents and the lowest marketable quality scores over time. Total chlorophyll and carotenoid content were slightly affected by leaf maturity and processing over time at storage temperatures lower than 5°C. Respiration rates were higher in immature than in mature and overmature leaves. Objective
color was not affected by leaf maturity but was influenced by storage temperature and fresh-cut processing over time. Fresh-cut processing and temperature hastened the senescence process by increasing the rate of overall deterioration and compositional degradation, as well as respiration rates. Mature fresh-cut leaves stored at 5°C in air + 15% CO2 atmosphere for 24 days had the highest marketable quality and compositional attributes, except for off-odors and total ammonia content, respectively, compared to leaves stored in air or air + 7.5% CO2. Control fresh-cut leaves had the lowest postharvest performance.
TO MY MOTHER AND GRANDMOTHER, MY GREATEST SOURCES OF UNCONDITIONAL LOVE, SUPPORT AND WISDOM
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1. INTRODUCTION

1.1 Kale as a Nutritious Leafy Green in Markets

Over the last two decades the consumption of fresh fruits and vegetables has increased as a result of changes in the consumption patterns of the U.S. population. This is a consequence of changes in demographics, higher incomes, more awareness and knowledge of the link between diet and health and a greater policy engagement regarding U.S. health issues, reflected in massive public campaigns that recommend and promote the consumption of these products (Bartlett et al., 2013; Cook, 2002, 2011). Along with these changes, the consumption of fruits and vegetables in “fresh-cut” or “minimally processed” format has also increased since the late eighties, when they first started to be present on the shelves of grocery stores due to their ease of preparation and convenience (Pollack, 2001).

According to the USDA, the term “fresh-cut” for leafy greens refers to:

*Fresh leafy green vegetables that have been altered from their natural form by cutting, dicing, peeling, slicing, chopping, shredding, coring, or trimming, with or without washing prior to being packaged for use by the consumer, foodservice industry, or a retail establishment* (U.S. Department of Agriculture, 2011).

One of the leafy green vegetables that has gained importance recently is kale (*Brassica oleracea* L. var. *acephala* DC.). Kale consumption has increased since 1997, when it initially started to appear in USDA statistics, reaching a total supply of 93.2 million pounds and 0.3 pounds per capita availability in 2010 (U.S.D.A., 2012).

The increasing information and available knowledge about kale’s nutritional content is certainly one of the reasons explaining this trend; however its consumption is still relatively low.
compared to other leafy greens, such as spinach and lettuce (Lesfrud et al., 2005; U.S.D.A., 2012).

Numerous studies have indicated an inverse relationship between the chemical compositions of vegetables from the Brassicaceae family and the risk of cancer (Peto et al., 1981; Steinmetz & Potter, 1996; Verhoeven et al., 1996; Wattenberg, 1992). Some of the most studied chemical compounds among Brassica vegetables are glucosinolates (Beecher, 1994; van Poppel et al., 1999) as well as other antioxidants, such as ascorbic acid, carotenes, tocopherol and phenolic compounds (Soengas et al., 2011).

Antioxidants are a group of enzymatic and non-enzymatic compounds that regulate the action of reactive oxygen species (ROS) that are generated during oxidative stress. Antioxidants act by delaying, inhibiting or preventing the oxidation of cellular components using two mechanisms: directly scavenging ROS or re-reducing targets that have been already oxidized (Hodges & DeLong, 2007). Interestingly, kale has been shown to have the highest total antioxidant activity among Brassica vegetables (Soengas et al., 2012).

Currently fresh kale is present in retail markets in different formats, such as whole intact leaves and fresh-cut leaves. ‘Winterbor’ (i.e. Curly kale, Scotch kale), ‘Lacinato’ (i.e. Tuscan kale, Black kale, Cavolo nero, Dinosaur kale) and ‘Red Russian’ (i.e. Ragged Jack kale) are the most widespread cultivars commercially in the U.S.

In terms of its format, fresh kale is present at retail in bunches of 6-20 intact whole leaves of 20-30 cm length, depending on the cultivar. At the same time, it is possible to find whole intact baby kale leaves of no more than 6-8 cm in mixed salads with other baby leafy greens, such as mizuna, spinach and lettuce, among others.

Fresh kale is also commercialized in fresh-cut or “minimally processed” format and it is possible to observe different types: “trimmed” kale leaves, where the petiole and basal area
have been removed and also “chopped” leaves of 1cm strips approx. In both formats kale can be alone or in combination with other vegetables as mixed salad.

Kale is harvested several times from the same plant and leaves can be harvested at different stages of maturity and development. This represents a problem in postharvest, due to the heterogeneous performance and quality of leaves at different stages of maturity, particularly those destined for fresh-cut processing.

1.2 Preparation for Market and Fresh-Cut Processing

For the fresh-cut process, the starting point is the harvest of leaves. This process is usually done manually, which has numerous advantages over machine harvest, such as the correct determination of leaf maturity, quality, minimization of mechanical damage and an accurate selection by person cutting (Thompson, 2002). This is especially important in kale, in which the plant is harvested several times during the production cycle as leaves mature.

After harvesting, intact kale leaves can be washed to remove soil, dirt, debris and other contaminants, especially if they will be commercialized in bunches (Thompson et al., 2002b; Raju et al., 2010). Kale is room cooled, vacuum cooled and forced-air cooled depending on the size of the operation and the diversity of products being grown (Thompson et al., 2002a).

For product intended for fresh-cut processing, the main idea is to minimize processing to retain fresh-like color, flavor and texture (Gunes & Dogu, 2010; U.S.D.A., 2005). Operations can be summarized as follows: after reception of the harvested leaves, which are generally a mixture of leaves at different maturity stages, all operations are done in cold, clean processing rooms at temperatures between 4-8°C.

Depending on format of the final product, leaves can be trimmed or chopped by rotating knives or by tearing into salad/size pieces. In this it is critical to mention the importance of using
sharp knives in the operation, which will contribute to improve the shelf life of the product. Once the product is cut, the next step in the processing is washing, usually with cold flowing water containing disinfectant, which removes sugars and other nutrients that promote bacterial and fungal growth and tissue discoloration as well. Cold water (below 5°C) also contributes to provide rapid cooling. Commonly, chlorine is used as disinfectant, with a residence time of 15-30 sec.; however water with 0.1% NaOCl results in a 10-fold reduction in aerobic plate count compared to fresh-cut kale that was not washed (Beaulieu et al., 1997; Cantwell & Suslow, 2002; Krasaekoopt & Bhandari, 2010). Passing the product over a vibration screen, centrifugation or other drying technique is used to remove excess of moisture that will contribute to microbial growth (Cantwell & Suslow, 2002; Krasaekoopt & Bhandari, 2010).

Dried fresh-cut product is weighted and can be directly packaged in plastic bags or rigid containers, alone or in combination with other products for salad mixes (Cantwell & Suslow, 2002; Krasaekoopt & Bhandari, 2010). Specific recommendations for kale establish that fresh-cut product in perforated (5.96 ± 0.35 mm² diameter holes with 6.68 cm² total surface area per sack) polyethylene (PE) bags become unacceptable due to yellowing and microbial spoilage after 3 days of storage at 4°C (39°F). Desiccation, yellowing and spoilage were unacceptable after 1 or 2 days at 20 or 10°C (68 or 50°F), respectively (Beaulieu et al., 1997).

On the other hand, whole leaves can also be directly packaged in plastic bags or rigid containers and finally both formats, fresh-cut and intact leaves are boxed, palletized and stored temporarily ("short term storage ") for further transport to food service outlets and/or retail markets (Cantwell & Suslow, 2002; Krasaekoopt & Bhandari, 2010).

Considering a standard storage period of 10 days, optimum conditions for kale are in a range of 0°-2°C and 90-98% of relative humidity (RH). Kale is moderately sensitive to ethylene,
therefore it is important to avoid storage next to products with moderate to high ethylene production rates (Rushing, 2004).

Kale is a leafy vegetable classified in the group of commodities with “very high” respiration rates (Kader, 2002a). Considering this and the significant damage caused by fresh-cut processing, compromises the development of packages that maintain kale leaves in good conditions until consumption. Modified atmosphere packaging (MAP) is an effective postharvest technique in prolonging the shelf life of several horticultural commodities. It consists in the modification of the gas concentration in the package atmosphere surrounding the commodity, typically involving the reduction of oxygen and/or elevation of carbon dioxide concentrations via the interaction between oxygen uptake (due to the respiratory process) and CO2 evolution of the packaged commodity (Kim et al., 2004).

The equilibrium of gas concentrations in MAP is affected by several factors that should be taken in consideration when packaging is designed, such as commodity weight, package film O2/CO2 transmission rate (amount of gas that passes through the package film, which is a porous material, over a period of time), respiring surface area and storage temperature. When the package film permeability has successfully matched the reparation rates of the product, a beneficial MAP can be determined and it will be able to extend shelf life by delaying senescence and deterioration. Conversely, when a packaging has not been successfully designed, oxygen may be depleted and carbon dioxide increased, thus leading to fermentative metabolism, anaerobic respiration and finally to hastening of the senescence process (Kim et al., 2004, 2005).

Currently for kale and other packaged fresh-cut vegetables, package surface area and commodity fill weight are frequently pre-determined to achieve and meet market conditions and
appeal, not taking in consideration the numerous factors affecting the dynamics of MAP (Kim et al, 2004).

1.3 Biology of Fresh-Cut Leafy Vegetables

Compared to conventional food processing, which extends the shelf life of fruits and vegetables, fresh-cut operations provide convenience and value added to consumers, but at the same time cause a faster physiological deterioration, biochemical changes, microbial susceptibility and degradation, and subsequently loss of shelf-life of the product, even when it has been slightly processed (Barth et al., 2004; Cantwell & Suslow, 2002; Garcia & Barrett, 2002; Rico et al., 2007; Varoquaux & Wiley, 1994).

The quality of fresh-cut products is directly related to a series of attributes, such as external and visual appearance (freshness, color, defects and decay), organoleptic characteristics (texture, turgidity, crispness, tissue integrity, flavor and aroma) and also nutritional value (minerals, vitamins, antioxidants, fiber) and safety aspects (absence of pathogens and chemical residues) (Francis et al., 2012; Piagentini et al., 2002).

Consumers choice is strongly correlated with appearance, therefore their satisfaction will affect subsequent and future purchases (Francis et al., 2012). Particularly in leafy vegetables, quality is significantly decreased with losses in green color due to chlorophyll breakdown as part of the senescence process (Koukounaras, 2009) and also by the production of undesirable gray-brown compounds, which can be enhanced by the presence of ethylene (Gaur et al., 2006; Heaton & Marangoni, 1996; Lipton, 1987; Piagentini et al., 2002). Consequently, it is of utmost importance to preserve the mentioned attributes at their peak, as well as their nutritional value (Garcia & Barrett, 2002).
Due to high perishability, high quality raw material is required for fresh-cut production (Cantwell & Suslow, 2002; Portela & Cantwell, 2001). U.S. Standards for Grades of fresh kale were established in 1934 and revised in 2005 and include uniformity of growth and color, trimming, freshness, and freedom from defect and decay (U.S.D.A., 2005).

According to Brecht (1995), the physiology of lightly processed fruits and vegetables is the physiology of wounded tissue. Therefore, the physiology of fresh-cut vegetables could be compared to one typically observed in plant tissues that have been exposed to stress conditions, because despite its state, fresh-cut product consists of tissue in a living, respiring physiological state (Ahmed & Alam, 2010).

Physiological changes experimented by wounded leafy tissues as a result of fresh-cut preparation include an increase in respiratory rate and ethylene production. Ethylene produced as a response of physical stress conditions, in this case wounded tissues, is sufficient in amount to affect adjacent tissues and induce a rise in respiration. However, vegetative non-climacteric tissue, such as lettuce and kale leaves, only experiment a transitory increase in ethylene production (Saltveit, 1999).

Higher respiratory rates in wounded tissue imply a more active metabolism and in consequence, a faster deterioration rate (Cantwell & Suslow, 2002). On the other hand, ethylene production as a response of wounding in vegetative tissue promotes a series of metabolic processes that accelerate senescence and deterioration (Brecht, 1995). Increased respiration rates, chlorophyll breakdown (leading to yellowing) and enhancement of phenylpropanoid metabolism by the action (increase in synthesis and activity) of the enzyme phenylalanine ammonia lyase (PAL) that triggers oxidative browning, especially cut-edges, are some of the greatest consequences wounding in fresh-cut preparation (Barth et al., 2004;
Lipid peroxidation is caused by enzymatic degradation and triggered by the production of reactive oxygen species (ROS) that act as signal messengers after wounding (Reyes et al., 2007; Rolle & Chism, 1987). At the same time, lipid peroxidation affects the integrity and function of membrane systems, including proteins, and also generates a variety of toxic compounds and secondary metabolites (Merzlyak & Hendry, 1994; Prochazkova & Wilhelmova, 2007).

Fresh-cut or wounded plant tissues, compared to intact, are directly exposed and in contact with the surrounding outside atmosphere, therefore water evaporation rate increases dramatically, which is favored by the large surface area of fresh-cut tissues (Barth et al., 2004; Brecht, 1995; Brecht et al, 2004; Garcia & Barrett, 2002; Watada et al., 1996). Moreover, increase in water loss is directly associated with changes in texture, wilting and decrease in vegetative tissue firmness due to drop of cell turgor as well as membrane integrity (Barrett et al., 2010; Toivonen. & Brummell, 2008).

One of the most critical aspects regarding physiological changes caused by fresh-cut processing is related to an increased susceptibility for microbial entry (Barth et al., 2004; Rico et al., 2007). Open cut surfaces release cell nutrients and substrates that are mostly available for bacterial and fungal growth, but also viruses (e.g. Hepatitis) and parasites (e.g. Giarda). Moreover, increased handling and potential use of dirty equipment during fresh-cut preparation can facilitate product contamination with human pathogens, such as Listeria, Salmonella and E.coli, among others (Cantwell & Suslow, 2002; Francis et al, 2012; Gil et al., 2009). Likewise, several microorganisms that constitute mesophilic microflora have been found in fresh-cut products, therefore is important to use low temperatures and adequate sanitation conditions during processing (Brecht et al, 2004; Watada et al, 1996).
1.4 Leaf Senescence

Leaves are by definition the ultimate photosynthetic organ. During its life span, a leaf undergoes three main phases of development, starting by a rapid expansion (phase 1), coupled with carbon and nitrogen import, and accompanied by rapid protein synthesis until the full photosynthetic capacity (phase 2) is reached. At this phase, the leaf becomes a metabolic and nutrient “sink” for the plant, contributing to carbon assimilation. This dynamic is maintained until internal (age and hormone levels) or external signals (temperature, shading, light, drought, nutrient deficiency and pathogen infection, among others) trigger the onset of “natural” senescence (phase 3) (Buchanan-Wollaston, 1997; Noodén et al., 1997; Wingler & Roitsch, 2008; Yoshida, 2003). In other words, natural leaf senescence process is triggered by the transition from sink to source, or when metabolic carbon assimilation is replaced by catabolism or degradation of chlorophyll and other macromolecules as well as nutrient recycling (Ansari & Chen, 2011; Biswal & Biswal, 1999; Gan & Amasino, 1997; Hörtensteiner & Feller, 2002; Lim et al., 2003; Soudry et al., 2005).

Natural leaf senescence is a developmentally programmed degeneration and “programmed cell death” (PCD) process (Buchanan-Wollaston, 1997; Lim et al., 2007; Woo et al., 2003), which comprises the final phase of leaf development and is controlled by multiple developmental and environmental signals that finally limit the life span or longevity of a leaf (Lim et al., 2003; Park et al., 2007).

During natural senescence, leaf cells undergo dramatic changes in metabolism and the sequential and ordered degeneration of cellular structures as well, beginning with chloroplast disassembly, accompanied by loss of photosynthetic activity, hydrolysis of macromolecules (e.g. chlorophyll) that have accumulated during the growth phase and massive remobilization of the hydrolyzed molecules to the growing parts of plants (Hörtensteiner, 2006; Prochazkova &
Consequently, leaf senescence is far from being a passive aging process, but an active and organized one culminating in the detachment of this organ in benefit of the whole organism, moreover an “altruistic” process (Biswal & Biswal, 1999; Gepstein, 2004; Lim et al., 2003).

There are several different patterns of natural plant senescence, such as overall or whole plant senescence, which includes monocarpic and polycarpic senescence; top senescence, occurring in geophytic plants and perennial herbs when aboveground or aerial parts senesce completely and new shoots appear at the beginning of the next season (Hikosaka, 2005; Kutbay, 1999; Noodén et al., 2003; Pogson & Morris, 2003); deciduous senescence occurring in deciduous plants or woody species that loss their leaves during the growing season before winter in preparation for the next growing season (Hikosaka, 2005; Koike, 2003; Pogson & Morris, 2003); progressive senescence where older leaves senesce as new leaves are produced; and bottom senescence, of storage organs during sprouting, leaving the terminal buds to overwinter (Noodén, 1988; Pogson & Morris, 2003).

Although this classification cannot be totally applied to harvested leafy vegetables, some mechanisms and metabolic pathways of harvested-induced senescence or “artificial senescence” are similar and common to those of natural deciduous senescence (Buchanan-Wollaston, 1997; Gan & Amasino, 1997; King & O'Donoghue, 1995; Koukounaras, 2009; Page et al., 2001; Pogson & Morris, 2003; Quirino et al., 2000) but also depict significant differences, such as the inability of metabolite movement and nutrient recycling on detached or harvested leaves (Pogson & Morris, 2003). Moreover, numerous stresses are represented by the detachment of leaves and include dehydration, wounding, removal of the supply of nutrients, hormones and energy, and also the inability of leaves to maintain homeostasis after detachment, because they are not adapted. Consequently this causes an interruption and
restriction of certain metabolic (particularly anabolic) processes and also activates and accelerates others, such as the senescence pathways (Pogson & Morris, 2003; Watkins, 2003).

The onset of substantial vegetable senescence is determined at the time when the limits of marketability (or appearance) of the vegetable are reached, also known as “storage” or “postharvest life”, which is variable depending on the species and cultivar (Pogson & Morris, 2003). However, a common feature of both types of senescence in leaves, natural and induced, is the degradation and disassembly of chlorophyll molecules that leave carotenoids exposed, showing the characteristic colors of senescent leaves (Ansari & Chen, 2011; Balazadeh et al., 2008; Bleecker, 1998; Takamiya et al., 2000).

Several studies have analyzed the differences between developmental and induced leaf senescence from a genetic perspective, and most of them have concluded that Senescence-Associated Genes (SAGs) respond in a similar way under the occurrence of natural and induced or artificial senescence; in other words, there is an overlap of SAGs between both processes (Nam, 1997). van der Graaff et al. (2006) performed transcriptome analysis of membrane transporters and hormone pathways in Arabidopsis thaliana leaves at three senescence conditions: first, using leaves at six successive developmental stages from a sink leaf stage to a late senescent stage (NS). Second, using individual leaves at darkening-induced senescence that were attached to the plant (DIS) and third, utilizing detached leaves and exposing them at dark-incubation (DET). The study concluded that the three senescence processes shared a significant number of regulated genes, but DIS and DET showed a two-fold lower number of regulated genes than NS. Moreover, there were substantial differences in terms of the expression of genes related to the remobilization of catabolites as well as hormone homeostasis, transporters, membrane proteins and signaling molecules, such as kinases.
Becker and Apel (1992) studied gene expression in barley leaves exposed to detachment and subsequent wounding; leaves from plants grown under drought stress; and flag leaves harvested at different weekly intervals, from anthesis until complete degradation. The results showed that only a minor part of the transcripts changes that were observed in dark-incubated detached leaves were connected to natural leaf senescence, whereas transcripts related to stress predominated.

Furthermore, Chang et al. (2003) compared the expression of a wheat RNase (nuclease protein that catalyzes the degradation of RNA) in naturally senescent flag leaves at different stages and dark-induced senescent leaves and concluded that it was down-regulated in both cases.

Conversely, when Buchanan-Wollaston et al. (2005) investigated differences in gene expression and signaling pathways among naturally senescing *Arabidopsis thaliana* leaves (developmental senescence); dark-induced senescing leaves and sucrose starvation-induced senescent leaves through transcriptome analysis, finding substantial differences. Both induced senescent conditions (darkness and starvation) presented gene expression patterns that were more similar between them than compared to natural senescing leaves, mostly due to the existence of sugar starvation that acted as the main initial senescence-signal in the former cases. On the other hand, genes involved in salicylic acid, jasmonic acid and ethylene pathways were shown to be necessary for expression of several genes in the course of developmental senescence. Furthermore, jasmonic acid and ethylene pathways are suggested to be involved in the regulation of dark-induced and sucrose starvation-induced senescence, but salicylic acid pathway appeared to be not involved. Finally, alternative pathways for nitrogen mobilization were present among different types of senescence.
In consequence, understanding the common and divergent mechanisms of both natural (developmental) and induced (artificial and harvest-induced) leaf senescence is relevant, in part because leaves are the main organ of consumption in many crop species. This could improve the control of conditions leading to leaf deterioration in terms of its compositional and sensory quality once it they are harvested. Furthermore, unrevealing the interaction between pre and postharvest factors and its impact on the performance of vegetable and particularly leafy products, can significantly contribute to the development of new or the improvement of current practices that can be applied commercially.

Finally, considering that the degree of greenness is a fundamental, and perhaps the most important quality aspect of leafy vegetables (Cantwell & Kasmire, 2002; Heaton & Marangoni, 1996); then understanding and controlling the diverse factors leading to loss of chlorophyll (and in consequence, yellowing increase), constitutes a crucial step in the improvement of the postharvest performance of these crops.

1.5 Quality and Postharvest Studies of Kale

The number of scientific publications and literature about kale has increased significantly in response to its increased consumption and popularity gained. A significant part of the research attention and endeavors have been focused on the nutritional content and benefits derived from consumption, emphasizing its role in the prevention of cardiovascular, degenerative diseases and diverse forms of cancer. Kurilich et al. (1999) performed analyses of carotene, tocopherol and ascorbate contents in edible tissues of 22 vegetables the Brassica family, including different varieties of broccoli, cabbage, cauliflower, kale and Brussels sprouts; and kale was the most potent source of antioxidants. Soengas et al. (2011, 2012) delved more into compositional analyses but came to similar conclusions about the nutritional value in kale.
Ayaz et al. (2006) detected fructose, glucose and sucrose as the major soluble sugars, and citric and malic acid as the major organic acids in kale leaves. The 16:0, 18:2n-6 (linoleic acid) and 18:3n-3 fatty acids were the most abundant fatty acids in leaves. The most abundant aminoacids were glutamic and aspartic acids. Additionally, Ayaz et al. (2008) identified 9 phenolic compounds in mature kale leaves and 10 in kale seeds, with ferulic and caffeic acids being the most abundant in leaves and sinapic acid in seeds. This research group went further and analyzed the antimicrobial activities of total phenolic compounds, observing that all of the fractions were effective against *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis* and *Moraxella catarrhalis*. Olsen et al. (2009) provided a more detailed analysis about phenolic compounds, tentatively identifying more compounds (three hydroxycinnamic acids and one flavonoid, quercetin-3-disinapoyl-triglucoside-7-diglucoside) than previously reported. In addition, 3 hydroxycinnamic acids and one flavonoid were tentatively identified for the first time as well.

The effect of different processing methods on nutritional content of kale has been extensively studied. Korus & Lisiewska (2011) investigated changes in vitamin C and polyphenol compounds in kale leaves after preliminary processing (blanching and cooking) and different methods of preservation by freezing and canning, comparing them with raw leaves without processing. The results showed that after raw consumption of kale leaves, which represented the control of the study, freezing is the best method of preservation, considering that the loss of nutritional compounds was lower than in the case of canning. After 12 months of storage, frozen products were also able to retain the highest nutritive compounds compared to canned products. Moreover, frozen products from blanched raw leaves contained the highest level of compounds, followed by frozen products obtained from cooked raw material and finally canned products.
Wang (1998) exposed kale leaves to heat treatment at 45°C for 30 min, resulting in a delay of yellowing, sugar losses and organic acids; preservation of good marketability and reduction of 25% total chlorophyll content, during subsequent storage for 7 days at 15°C. Similarly, Lisiewska et al. (2008) evaluated the level of aminoacids and the quality of protein in fresh and cooked kale leaves and in two type of frozen product prepared for consumption after 12 months stored at -20°C. In the latter, the first method, or “traditional”, kale was blanched before freezing and then cooked. In the second or “modified” method, kale was cooked before freezing and then defrosted and heated in a microwave oven. The results showed that cooked kale leaves presented a reduction in the totality of analyzed aminoacids, except for methionine that was maintained. Cooked leaves contained 78% of total aminoacid content found in fresh leaves, while traditional and modified methods presented 76 and 78%, respectively. The proportion of essential aminoacids to total aminoacids was around 43% in all treatments.

Korus (2012a) performed a similar study and the results were consistent with the experiment previously described. Sikora & Bodziarczyk (2012) compared the chemical composition and antioxidant activity of raw and cooked (boiling water, kept at 100°C for 12-15 min) kale leaves. The results showed a significant decrease in antioxidant activity, vitamin C, and polyphenolic compounds in cooked kale, whereas β-carotene remained relatively stable. Losses in macronutrient content, nitrites and nitrates were also detected.

Conversely, Hagen et al. (2009) stored curly kale leaves at 1°C for 3 and 6 weeks and compared them with plants remaining in the field regarding compositional properties. The results exhibited no effect of cold storage on antioxidant capacity, total phenols and flavonol content but vitamin C and soluble sugars were reduced. Furthermore, the most significant changes were observed in plants that remained in the field for additional 6 weeks, including many frost days, where all compositional attributes were significantly reduced, except for soluble sugars and dry matter, which increased.
The effect of different storage treatments, including modified atmosphere packaging and controlled atmospheres, on the postharvest performance of kale leaves was also analyzed by several research groups. Carnelossi et al (2002) investigated the effect of temperature (1, 5 and 10°C) and type of packaging (high and low permeability) in postharvest performance of fresh-cut kale leaves stored for 10 days. Results showed that kale stored at 5°C in high permeability pack, presented a good quality for 10 days in terms of chlorophyll, carotenoids, vitamin C contents and sensory quality. Products stored at 1 and 5°C in low permeability packaging maintained a constant chlorophyll and carotenoid level until day 5, but vitamin C decreased over time. Finally, at 10°C, chlorophyll and vitamin C levels were significantly reduced in all treatments, and sensory quality decreased considerably by day 10.

Fonseca et al. (2003) studied the benefits and risks of low oxygen and high carbon dioxide levels in fresh-cut kale for the potential utilization of modified atmosphere packaging (MAP). For this aim, respiration rate, sensory attributes, color changes, chlorophyll, ascorbic acid and water content were assessed. Low oxygen tolerance (1, 2, 3 or 21% v/v O2 without CO2) was tested, maintaining the quality of fresh-cut leaves longer and showing no induction of anaerobic respiration at low O2 atmospheres. High carbon dioxide tolerance (0, 10, 15, or 20% v/v CO2 plus 21% v/v O2) was tested and also extended the shelf life, exhibiting no symptoms of CO2 injury. Combinations of low O2 and high CO2 (1 or 2% v/v O2 plus 15 or 20% v/v CO2 and air as control) were analyzed, showing no significant differences among them, however an atmosphere of 1-2% v/v O2 plus 15-20% v/v CO2 extended the shelf life at 20°C to 4 days, compared to 2 days in air storage (control conditions).

Moretti et al (2003) evaluated the postharvest performance of fresh-cut kale leaves stored under two controlled atmospheres (CA) conditions: 3% O2 plus 4% CO2/balance N2 and 5% O2 plus 5% CO2/balance N2; and a control (normal air) at 5°C and 95±2% RH for 6 days. Results concluded that CA 3% O2 plus 4% CO2/balance N2 presented the highest levels of
total chlorophyll, total vitamin C and organic acid content, followed by 5% O2 plus 5% CO2/balance N2 and finally the control, however, total soluble solids content was not significantly affected.

Brecht et al. (2003) designed a combination MAP/CA system able to generate an optimal atmosphere for retail display conditions but at the same time interacting with an optimal CA, thus producing a desired atmosphere within the packages during kale transportation. In conclusion, a perforation-mediated MAP for fresh-cut kale that generates an atmosphere of 2% O2 plus 17% CO2 at 20°C will produce the optimal atmosphere of 1% O2 plus 10% CO2 at 1°C if stored in a surrounding CA of 5% CO2 plus 7.1% CO2.

Similarly, Kim et al. (2004) developed a MAP system for fresh-cut kale at two varieties (white and violet), then evaluated the effect of film oxygen transmission rate (OTR, expressed in pmol s⁻¹ m⁻² Pa⁻¹) on package atmospheres and postharvest performance of the product. Results suggested that packages with 16.6 and 21.4 OTR films reached the desired O2 and CO2 levels on day 10, and up to this day, product exhibited the highest quality scores. On the other hand, packages with 8.0 OTR film showed a rapid depletion of O2 and accumulation of CO2, generating unacceptable quality. Packages with 29.5 OTR film exhibited undesirable product quality as well. Additionally, white kale presented higher respiration rates and lower quality scores than violet kale, but both varieties showed good quality during the 25 day storage period under the optimal atmosphere conditions.

Kobori et al. (2011) processed fresh-cut leaves, packed them in passive modified atmosphere and stored them at 3 conditions: 1°C in dark and 11°C with or without light exposure in order to determine gas composition, sensory attributes, flavonol and carotenoid content. Results showed that passive modified atmosphere packaging with refrigeration extended the shelf life of fresh-cut kale. Flavonols quercetin and kaempferol were stable during
storage, increasing under light exposure; carotenoids neoxanthin and violaxanthin were stable at 1°C in the dark, but lutein and β-carotene slightly decreased. At 11°C in the dark, the 4 major measured carotenoids decreased but under light, results were heterogeneous.

The effect of leaf maturity at harvest and its interaction with other storage and agronomic variables is other important issue. Lefsrud et al. (2007) investigated nutritional differences (chlorophyll a and b, lutein and β-carotene) of kale leaves grown in a controlled environment and harvested at different times: <1 week (young leaves), 1-2 weeks (immature leaves), 2-3 weeks (mature leaves), 3-4 weeks (fully developed weeks) and >4 weeks (senescing leaves). There were significant differences for all pigments during leaf ontogeny: maximum pigment accumulation was reached between weeks 1-3 of leaf age. The highest concentration of lutein was recorded in 1-2 week old leaves, whereas the rest of the pigments presented their highest levels at 2-3 weeks old leaves. Additionally, mature fully expanded leaves accumulated higher carotenoid levels than immature tissue, with senescing leaves exhibiting the lowest carotenoid contents and mature leaves presenting the highest carotenoid content.

Similarly, Acikgoz (2011) studied the effect of harvesting stages (rosette stage, budding stage and flowering/blooming stage) on mineral (nitrogen, iron, phosphorous, potassium, calcium, magnesium, manganese, copper and zinc), vitamin C and crude protein contents of kale leaves. In conclusion, the highest values for vitamin C were reached by harvest at the flowering/blooming stage; the highest values for crude protein, N and Fe were reached by the rosette stage and the highest values for the remaining elements were reached by harvesting at the budding stage.

de Acevedo & Rodriguez-Amaya (2004) analyzed the influence of maturity (young and mature leaves from the same bunch of kale), season (summer and winter), farm practices (organic and conventional) and minimal processing (marketed processed kale and minimally
processed kale) on carotenoid content of kale leaves. The β-carotene and lutein levels were significantly higher in mature leaves from conventional farms; violaxanthin presented significantly high levels in young leaves whereas neoxanthin had no significant differences at both maturity stages. In kale leaves from the organic farm, carotenoid levels showed no significant differences between maturity stages. Carotenoid contents of marketed processed kale were significantly higher in summer than in winter, except for β-carotene in which there were no differences related to the season. In minimally processed kale leaves stored at 5 days at 7-9°C, all carotenoids levels significantly decreased.

Korus (2011) studied vitamin C, L-ascorbic acid, polyphenols, antioxidant activity, peroxidase (POD), polyphenol oxidase (PPO) and catalase (CAT) in 3 varieties (Redbor F1, ‘Winterbor’ F1 and Medium High Green Curly) of kale at 3 maturity stages. Redbor variety presented the highest contents of all the measured compounds, moreover leaves at the second maturity stage had more vitamin C, polyphenols and antioxidant activity than those at the first stage. Leaves at the third maturity stage had a lower vitamin C content than those at the second stage, but higher than the first stage; and a higher polyphenol concentration and antioxidant activity than leaves at the second stage. On the other hand, PPO activity increased with the age of the plant, but no significant correlation was detected between maturity stages and other enzymes activities.

Different agronomic practices affect composition, yield and other features of kale. Eppendorfer (1996) reported the effect of nitrogen fertilization and phosphorous and potassium deficiency in terms of dry matter yield, nitrate and aminoacid content. Korus (2010) analyzed the effect of cultivar (‘Winterbor’ F1, ‘Redbor F1’ and ‘Medium High Green Curly’) and 3 harvest dates on crop yield and plant morphological features. Kopsell et al. (2003) investigated the influence of increased sulfur fertility levels on the accumulations of macronutrients (sulfur, magnesium and calcium), chlorophyll (a and b) and carotenoid (lutein and β-carotene) content;
and the production of glucosinolates and S-methylcysteine, two sulfur compounds with medicinal value but responsible for undesirable flavors according to consumers. Furthermore, Lefsrud et al. (2005) determined the effects of air temperature on chlorophyll (a and b), carotenoid (lutein and β-carotene), macro and micronutrient accumulation and biomass production (fresh weight and dry weight) in kale.

Kopsell et al. (2007) studied the influence of different photoperiods (6, 12, 16 or 24 hours, continuous) to determine changes in the accumulation of biomass (fresh and dry weight), chlorophyll (a and b) and carotenoid (lutein and β-carotene) on kale. Afterwards Kopsell et al. (2007) followed the same scheme and analyzed the effects of N concentration and form in chlorophyll (a and b) and carotenoid (lutein and β-carotene) concentration of leaf tissues and biomass production (fresh weight and dry weight) of kale. Likewise, Ligor & Buszewski (2012) determine the level of lutein on kale leaves under different growing conditions: low and high temperature, drought stress, saline stress and UV radiation.

Equally important, the influence of different kale cultivars has also been documented in the literature. Mercadante & Rodriguez-Amaya (1991) detected differences in the carotenoid (β-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin) and vitamin A content of kale leaves due to cultivars (‘Manteiga’ and ‘Tronchuda Portuguesa’), seasonal variations (winter and summer) and farming practices (organic and conventional). Furthermore, Kopsell et al. (2005) compared 22 kale cultivars and selections that were grown over 2 years in terms of their elemental accumulations on leaf tissue. Significant variability (2.24-fold on average) was found in elemental accumulation among the cultivars and selections. Moreover, significant year-to-year variability was detected for calcium, magnesium, iron and zinc accumulation.
Zietz et al. (2010) determined the influence of genotype (8 cultivars, including red and green kale as well as old, traditional and hybrid cultivars) and climatic factors (temperature and global radiation level) on the phenolic content and antioxidant activity of kale leaves.

In conclusion, papers about kale have been present in the scientific literature since the early 90's. There was an abrupt increase from the year 2000, which is consistent with the higher consumption of this leafy vegetable and at the same time, a greater need for increase the existent knowledge, especially about nutritional value and how to maintain it and delay its deterioration in postharvest.

The main objective of this study was to characterize the senescence process of kale leaves in postharvest by integrating different factors simultaneously. In order to achieve this goal, different experiments were designed and specific objectives were established: (1) To determine the impact of leaf maturity at harvest in combination with fresh-cut processing and temperature on the postharvest performance and senescence process of kale leaves obtained from two cultivars and different commercial sources. (2) To determine the impact of controlled and modified atmospheres in the postharvest performance and senescence process of mature kale leaves in fresh-cut format at different storage temperatures. (3) To develop correlations among indicators of the postharvest performance and senescence process of kale leaves, such as marketable quality attributes, color by destructive and non-destructive techniques, respiration and chemical composition.
2. MATERIALS AND METHODS

All experiments and analyses were performed in Mann Laboratory, Department of Plant Sciences, University of California, Davis between September 2011 and May 2013.

2.1 Impact of Processing, Leaf maturity and Temperature on the Postharvest Performance of Kale Leaves

2.1.1 Experiment 1: Mature intact leaves at three storage temperatures

Kale cv. ‘Winterbor’ was purchased from General Produce on March 26, 2012; product was from Church Brothers LLC, Salinas; it was packed as 24 bunches in waxed cartons and it had been hydrocooled and then top-iced with a paper separating product from the ice. Product was transported under cool conditions to the laboratory, ice was dumped and bunches were placed in plastic bags to prevent water loss and held at 0°C room until used.

Mature kale leaves were stored at 0, 10 and 20°C and then evaluated at 0, 3 and 6 days for objective color by 2 instruments: Konica Minolta spectrophotometer (CM-2500c), also known as portable spectrophotometer, and Konica Minolta colorimeter (Chroma Meter CR-300). One replication consisted of 6 leaves. A total of 4 replications were used.

Chlorophyll determination (composition analysis) was made by spectrophotometric assay at 0, 3 and 6 days. For this analysis, 4 bags were randomly selected at each time. One bag was equivalent to one replication and contained 4-6 leaves. Total chlorophyll content was also measured non-destructively by Minolta SPAD Chlorophyll Meter (SPAD 502 DL Plus). One replication consisted of 6 leaves. A total of 4 replications were used, except for 0°C and 10°C at day 6, with 1 and 2 replications, respectively.
2.1.2 **Experiment 2: Intact and fresh-cut leaves at three maturity stages and three storage temperatures**

Kale leaves cv. ‘Winterbor’ were harvested at three maturity stages: immature, mature and overmature in a field of Bruce Church Company in San Juan Bautista CA and placed in polyethylene bags in coolers with ice for transport to the laboratory on August 30, 2012.

Maturity stage criterion was based on leaf size, color and appearance. Immature leaves were the youngest and had not reached full expansion. They presented a lighter green color, length range between 11 and 17 cm and width range between 5 and 8 cm. Mature leaves had a length range between 18 and 27 cm, a width range between 12 and 17 cm. Some of them had not reached full expansion and others were estimated to be fully expanded but without visible symptoms of senescence. Overmature leaves were the oldest and exhibited some visible symptoms of senescence. They presented a darker green color; length range between 28 and 34 cm, a width range between 14 and 20 cm and were fully expanded.

Petioles were removed from leaves at both maturity stages. A group of leaves was maintained in the intact state and another group was fresh-cut processed: upper half was cut into about 1 cm strips across the midrib with a sharp stainless steel knife. Both types of leaves were washed in chlorinated water (5:1 ratio of solution to product weight; 50 ppm sodium hypochlorite solution adjusted to pH 7.0), shaken, drained on clean towels, placed in plastic unsealed polyethylene bags on trays and overwrapped with a larger bag to prevent water loss during storage at 0, 5 and 10°C during 6 days.

Respiration (CO2 concentration) was measured on a daily basis. In product stored at 0°C, respiration was recorded for 29 days; at 5°C for 18 days and in product at 10°C for 11 days. Three replications were used, except for intact immature leaves stored at 0°C, where 2 replications were used. One replication consisted of one sealed container with 6-8 kale leaves.
2.1.3 **Experiment 3: Intact and fresh-cut leaves at two maturity stages**

Kale cv. ‘Winterbor’, commercially packed and liquid iced was obtained from General Produce on May 23, 2012. Product was from Boskovich Farms, Oxnard and had been harvested on May 22. Product was transported in an air-conditioned van to the lab; ice was removed and the bunched product was transferred to large plastic bags in trays and held at 0°C until experimental setup on May 25.

Leaves were separated in two maturity stages (immature and mature) using the same criterion than in Experiment 1.

Petioles were removed from leaves at both maturity stages. A group of leaves was maintained on intact state and other group was fresh-cut processed, washed, manually centrifuged and placed in bags as in Experiment 2. They were stored at 7.5°C for up to 12 days.

Product was sampled for marketability analysis (Overall visual quality, yellowing and decay/deterioration) and composition (chlorophyll and carotenoids; antioxidant capacity and ammonia content) at 0, 4, 8 and 12 days. Four bags were randomly selected at each time evaluation. One bag was equivalent to one replication and contained 4-6 leaves.

Objective color, expressed in L*, a* b*, Chroma and Hue components, was measured with Konica Minolta colorimeter (Chroma Meter CR-300) at 0, 4, 8 and 12 days in intact and fresh-cut leaves. On intact leaves, one replication consisted of 4 leaves. A total of 4 replications were used. On fresh-cut leaves, one replication consisted of 4 strips. A total of 4 replications were used.
2.1.4 **Experiment 4: Intact and fresh-cut leaves at three maturity stages and two storage temperatures**

Kale leaves cv. ‘Winterbor’ at three maturity stages: immature, mature and overmature were harvested in the same field of Bruce Church Company in San Juan Bautista CA on August 23, 2012, placed in plastic bags in coolers with ice, transported to the lab and bags were stored at 0°C for 20 hours until August 24.

Maturity stage criterion was based on leaf size, color and appearance as in Experiment 2. Petioles were removed from leaves at all maturity stages. A group of leaves was maintained in the intact state and other group was fresh-cut processed, washed, manually centrifuged and placed in bags as described in Experiment 2. Intact and fresh-cut leaves were stored at 0 and 5°C.

Product was sampled for marketability analysis (off-odors, overall visual quality, yellowing, decay/deterioration and discoloration/browning) and composition (chlorophyll and carotenoids; ammonia and malondialdehyde content) at different time intervals depending on the storage temperature. Product at 0°C was evaluated at 0, 14, 28 and 42 days; product at 5°C was evaluated at 0, 14, 21 and 28 days. Four 4 bags were randomly selected at each time evaluation. One bag was equivalent to one replication and contained 4-6 leaves.

2.1.5 **Experiment 5: Fresh-cut leaves at three maturity stages and two storage temperatures**

Kale leaves cv. ‘Lacinato’ at three maturity stages: immature, mature and overmature were harvested from a field of San Miguel Produce in Oxnard CA on September 26, 2012, placed in unsealed plastic bags and transported in coolers with ice to the Mann Lab where they were stored at 0°C overnight.
Maturity stage criterion was based basically on leaf size. Immature leaves were the youngest and had not reached full expansion. They presented a length shorter than 20 cm and width inferior to 4 cm. Mature leaves had not reached full expansion and presented a length range between 20 and 30 cm and a width range between 4 and 5 cm. Overmature leaves were the oldest, presented a length longer than 35 cm and a width superior to 5 cm. They were fully expanded and some presented slight visible signs of leaf senescence.

On September 27, leaves fresh-cut processed washed, manually centrifuged, placed in bags as in Experiment 2 and transferred to 0 and 5°C rooms.

Product was sampled for marketability analysis (off-odors, overall visual quality, yellowing, decay/deterioration and discoloration/browning), composition (chlorophyll and carotenoids; ammonia and malondialdehyde content) and objective color at different time intervals depending on the storage temperature. Product at 0°C was evaluated at 0, 14, 28 and 42 days; product at 5°C was evaluated at 0, 14, 21 and 28 days.

For marketability and composition analyses, 3 bags were randomly selected at each time evaluation. One bag was equivalent to one replication and contained 4-6 leaves.

Objective color, expressed in L*, a* b*, Chroma and Hue components, was measured with Konica Minolta colorimeter (Chroma Meter CR-300) and Konica Minolta spectrophotometer (CM-2500c), also known as portable spectrophotometer. One replication consisted of 4 strips. A total of 3 replications were used.

Respiration (CO2 concentration) was measured on a daily basis. Product stored at 0 and 5°C was analyzed for 38 and 24 days, respectively. Three replications were used, except for immature leaves stored at 0°C, where 2 replications were used. One replication consisted of one sealed container with 6-8 kale leaves.
2.2 Impact of Modified and Controlled Atmospheres on the Postharvest Performance of Kale Leaves

2.2.1 Experiment 6: Fresh-cut mature leaves stored in air or CA

Mature kale leaves cv. ‘Winterbor’ were harvested in the same field of Bruce Church Company in San Juan Bautista CA and placed in polyethylene bags in coolers with ice for transport to the laboratory on August 30, 2012. Leaves were fresh-cut processed, washed, manually centrifuged and placed in bags as in Experiment 2. Bags were transferred to 5°C and held in three different controlled atmospheres (CA): Air (control); Air+7.5% CO2 and Air+15% CO2.

Product was sampled for marketability analysis (off-odors, overall visual quality, yellowing, decay/deterioration and discoloration/browning) and composition (chlorophyll and carotenoids; ammonia and malondialdehyde content) at 0, 10, 14, 20 and 24 days. Four bags were randomly selected at each evaluation time. One bag was equivalent to one replication and contained 4-6 fresh-cut kale leaves.

2.2.2 Experiment 7: Fresh-cut leaves stored in MAP at three temperatures

Kale cv. ‘Winterbor’ was obtained from the fresh-cut processor Harvest Sensations (1205 Wholesale St., Los Angeles, CA 90021) on September 3, 2011; 6 boxes of 10 8 oz packages (cut kale plus carrot and red cabbage; lot 245-62, kale from Kenter Canyon Farms, Ventura; BIUD 9/16).

Product was cut, washed, centrifuged and packaged (microperforated packaging) in modified atmosphere (MAP) on September 2 and held at 1-2°C until picked up on September 3. Product was transported to the lab in coolers with wrapped gel ice packs and arrival
temperatures after 6 hours were 0-5°C. Product was placed at 0°C until transferred to the 0, 5 and 10°C rooms.

Six bags were prepared with an application of silicon to serve as a port to measure O2 and CO2 concentrations on a daily basis for 20 days. One bag was equivalent to one replication.

Product was sampled for marketability analysis (Off-odors, overall visual quality, yellowing, decay/deterioration and discoloration/browning) at different time intervals depending on the storage temperature. Product at 0°C and 5°C was evaluated at 0, 8, 12, 16, and 20 days; product at 10°C was evaluated at 0, 4, 8, 12 and 16 days. At each evaluation time, 3 bags were randomly selected. One bag was equivalent to one replication. Only fresh-cut kale was evaluated.

2.3 Marketability Evaluations

Subjective evaluations were performed on kale leaves using hedonic scales based on different quality parameters. Bags containing were removed from storage and immediately evaluated at ambient temperature. Each bag received one score per quality parameter (Cantwell & Portela, 1998; López-Gálvez et al., 1997). Quality parameters were described as follows:

Off-odors were scored on a 1 to 5 scale, where 1 = No off-odor; 2 = Slight off odor; 3 = Moderate; 4 = Moderately severe and 5 = Severe off odors.

Overall visual quality was scored on a 9 to 1 scale, where 9 = Excellent, fresh appearance; 7 = Good; 5 = Fair (Limit of marketability); 3 = Fair (Usable but not salable); 1=usable. Intermediate numbers were assigned where appropriate.
Yellowing was scored on a 1 to 5 scale, where 1 = No yellowing; 2 = Slight loss of greenness or slight yellowing, but product salable (<2% affected); 3 = Moderate loss of greenness/yellowing, product usable but at limit of salability (<5% affected); 4 = Moderately severe (5-15% affected); 5 = Severe, unusable (>15% of leaf surfaces with yellowing).

Decay or visible deterioration. Named like this since initial decay is often not distinct but surface is showing deterioration. It was scored on a 1 to 5 scale, where 1 = No decay; 2 = Slight decay, but product salable (<2% affected); 3 = Moderate decay, product usable but not salable (<5% affected); 4 = Moderately severe (5-15% affected); 5 = Severe, unusable (>15% of leaf surfaces with decay).

Discoloration and/or browning on edge cut was scored on a 1 to 5 scale, where 1 = No discoloration; 2 = Slight discoloration, but product salable (<2% affected); 3 = Moderate discoloration, product usable but not at limit of salability (<5% affected); 4 = Moderately severe (<15% affected); 5 = Severe, unusable (>15% of leaf with discoloration).

2.4 Objective Color and Non-destructive Chlorophyll Estimation

Two different instruments: Konica Minolta spectrophotometer (“portable spectrophotometer”) (CM-2500c) and Konica Minolta colorimeter (Chroma Meter CR-300) determined objective color. The viewing angle for both instruments was CIE 2° Standard Observer. Independent of the light environmental conditions, measurements were made in a three-dimensional color space using L* a* b* scale, where L* represented “lightness” or a range between black to white; a* represented a range of colors between green to red and b* represented a range between blue to yellow. Chroma (degree of strength or intensity of a color) and Hue angle (color) were calculated as \((a^* + b^*)^{1/2}\) and \(\tan^{-1}(b^*/a^*)\), respectively (McGuire, 1992).
Measurements were made on the upper right quadrant of each leaf in the case of intact leaves. On fresh-cut leaves, measurements were made on the right quadrant of each strip.

Chlorophyll determination has been traditionally made by methods that involve the extraction of leaf materials and further use of spectrophotometry. Although these techniques have been used by numerous researchers for several years, they are not entirely straightforward and require the destruction of plant material. In consequence, the development and use of non-destructive optical methods based on the absorbance of light by leaves is more widespread and accepted nowadays. One of these methods consists in the use of an electronic device, a Chlorophyll meter (Chl SPAD meter) that uses two-light emitting diodes (650 and 940 nm) and a photodiode detector to sequentially measure transmission through leaves of red and infrared light (Markwell et al., 1995; Udling et al., 2007). The functioning of SPAD meter is based on the quantification of relative concentrations and proportion of pigments in leaves, and the absorbance predicted by Beer’s Law, allowing establishing correlations with total amount of chlorophyll obtained by spectrophotometry (Monje & Bugbee, 1992; Richardson et al., 2001, Udling et al., 2007).

Minolta SPAD Chlorophyll Meter (SPAD 502 DL Plus) was used to indirectly measure total chlorophyll content on intact and fresh-cut leaves. On intact leaves, measurements were made on the upper right quadrant of each leaf. On fresh-cut leaves, measurements were made on the right quadrat of each strip.

2.5 Composition Analyses

Different bags containing kale leaves were selected at each evaluation time to record changes in composition, such as chlorophyll and carotenoids, ammonia, malondialdehyde and total antioxidant capacity. Midribs were removed, leaves were finely chopped with a sharp stainless steel knife and a 4 g subsample was held at -20°C in falcon tubes until analysis.
2.5.1 Chlorophyll and carotenoids

Samples were homogenized (Ultra Max T25 Basic homogenizer by Ika-Werke) for 1.5 min at 17500 rpm adding 12 ml acetone 80% (10 mg MgCO$_3$ in 1000 ml 80% acetone) and then centrifuged (IEC Clinical centrifuge Model CL by International Equipment Company) at 3500 rpm for 5 min. The supernatant was moved in a 50 ml falcon tube, while the precipitated was extracted two more times. The resulting supernatants were combined and massed up to 50 ml with acetone. The final supernatant was 10 times diluted (10x dilution).

Chlorophyll and carotenoids were measured at 663.2, 646.8 and 470 nm (Shimadzu spectrophotometer UV-1700) and acetone 80% (10 mg MgCO$_3$ in 1000 ml 80% acetone) was used as a blank. Calculations were made according to Lichtenthaler (1987).

2.5.2 Antioxidant capacity (FRAP method)

Samples were homogenized (Ultra Max T25 Basic homogenizer by Ika-Werke) for 1.5 min at 17500 rpm adding 26 ml methanol 100%. 1 ml aliquot was transferred to an eppendorf tube and centrifuged (Microfuge 18 centrifuge) for 15 min at 14000 rpm at 4°C. Then 0.2 ml supernatant was transferred to test tube and 0.8 ml methanol 100% were added (5x dilution). 0.05 ml aliquot was added to 4 ml FRAP solution, shaken vigorously and incubated for 30 min at 37°C.

Ferric Reducing Antioxidant Potential (FRAP) solution consisted of a mixture of 300 mM acetate buffer (pH 3.6); 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 30 mM FeCl$_3$$\cdot$6H$_2$O in a ratio 10:1:1, respectively.

Antioxidant capacity was measured at 593 nm against a reagent blank (FeSO$_4$$\cdot$7H$_2$O) in a spectrophotometer (Shimadzu spectrophotometer UV-1700). A standard curve was made to
determine the total potential of antioxidants in samples, expressed in mmole Fe$^{2+}$/100g FW (Benzie, 1999; Thaipong et al., 2006).

2.5.3 Ammonia

Samples were homogenized (Ultra Max T25 Basic homogenizer by Ika-Werke) at 17500 rpm for 1.5 min adding 16 ml distilled water and 1.5 ml were transferred to an eppendorf tube. Tubes were centrifuged (Microfuge 18 centrifuge) for 15 min at 14000 rpm at 4°C. Ammonia concentration was determined by taking 0.5 ml aliquots and adding 2.5 ml phenol containing nitroprusside and 2.5 ml alkaline hydrochlorite. Tubes were shaken vigorously and incubated at 37°C for 15 minutes.

Ammonia was measured 635 nm (Shimadzu spectrophotometer UV-1700) against a reagent blank (phenol plus nitroprusside, alkaline hypochlorite and water). A standard curve was made to determine the concentration of ammonia in samples, expressed in mg NH$_4$/g FW. Some of the samples had to be diluted (20x) due to their high ammonia content (Beecher & Whitten, 1970; Weatherburn, 1967).

2.5.4 Malondialdehyde (MDA)

MDA was determined by a method modified from Mendes et al. (2009). Samples were homogenized (Ultra Max T25 Basic homogenizer by Ika-Werke) at 17500 rpm for 1.5 min adding 8 ml 7.5% trichloroacetic acid (TCA) solution, filtered with miracloth and centrifuged (Beckman J2-MI centrifuge) for 15 min at 10000 rpm. One ml aliquot was taken and added 1 ml thiobarbituric acid (TBA) 20 mM. Capped tubes were shaken vigorously and incubated at 100°C for 60 minutes, ice-cooled for 1 min and centrifuged (Microfuge 18 centrifuge) for 15 min at 14000 rpm at 4°C.
MDA was measured at 530 nm (Shimadzu spectrophotometer UV-1700) against a reagent blank (TBA 20mM in 7.5% TCA). A standard curve was made to determine the concentration of malondialdehyde in samples, expressed in nmol TEP/g FW (Mendes et al., 2009, Heath & Packer, 1968).

2.6 Gas Analysis

In Experiment 7, periodic measurements of oxygen and carbon dioxide were carried out by injection of 1mL gas samples from a sample port on the packaged kale into an infrared analyzer for CO2 (Model PIR-2000, Horiba Instruments Inc., Irvine CA) in series with an electrochemical oxygen analyzer (Model S-3A, Applied Electrochemistry Inc., Sunnyvale CA). Appropriate standard concentrations were used for calculations (9.913% for O2 and 9.992% for CO2).

In Experiments 2 and 5, respiration (or carbon dioxide production) was measured based on the dynamic or “flow through” system. Intact and fresh-cut kale leaves were weighed and placed inside sealed plastic containers connected to a humidified air stream (approx.95% Relative Humidity). A flowboard using capillary tubes as flow meters was used to control flow rates. Carbon dioxide concentration was measured by taking 1 ml gas samples from a sample port on the sealed plastic containers into an infrared analyzer for CO2. A standard of 0.5136% CO2 was used for calibration and the difference between inlet and outlet carbon dioxide concentrations was used for calculation of the respiration rates (Kader & Saltveit, 2002; Saltveit, 2004).

2.7 Statistics

Among experiments, different replications were used (either 3 or 4). Two replications were used in exceptional cases that were previously reported.
All statistical analyses were performed by SAS software Version 9.3 of the SAS System for Windows 64 bits. SAS Institute Inc., Cary, NC, USA. Analyses were carried out in a complete randomized factorial design and analysis of variance (ANOVA) was performed. For mean comparison, Tukey’s test and Least Significant Difference (LSD) were used with α=0.05.

For gas analyses (CO2 and O2), repeated measured analysis was performed.

Multiple line/scatter plots and vertical bar charts were graphed using SigmaPlot version 12.0, (Systat Software, Inc., San Jose CA, USA, www.sigmaplot.com).
3. RESULTS

The storage quality of intact or fresh-cut kale leaves at different stages of maturity was evaluated at a range of temperatures (0 to 10°C) and in air or modified atmospheres. Kale leaves were harvested and obtained from two cultivars (‘Winterbor’ and ‘Lacinato’) and different commercial sources.

3.1 Impact of Processing, Leaf maturity and Temperature on the Postharvest Performance of Kale Leaves

3.1.1 Experiment 1: Mature intact leaves at three storage temperatures

Mature intact kale leaves cv. ‘Winterbor’ were stored at 0, 10 and 20°C for 6 days (Figure 1).

3.1.1.a Objective color and non-destructive chlorophyll estimation

Changes in color were recorded by two instruments: colorimeter and portable spectrophotometer (Figure 2, Table 1). In leaves stored at 0 and 10°C, L* value remained relatively constant over time; however in leaves at 20°C there was a significant increase after 6 days of storage. The R² correlation between both instruments reached 0.78 (Figure 3).

For Hue angle, the colorimeter and spectrophotometer showed that at 0 and 10°C it remained relatively constant over time; however in leaves at 20°C there was a notable decrease after 6 days of storage (Figure 2). The R² correlation between both instruments reached 0.84 (Figure 4).

The highest value for a* component was recorded in leaves store at 0°C stored for 6 days, and the lowest one in leaves at 20°C stored for 6 days as well (Table 1). The b* and
Chroma components were relatively homogeneous, with the highest one recorded in leaves at 20°C stored for 6 days.

Chlorophyll was estimated non-destructively by a SPAD meter (Figure 5). Values exhibited a similar trend compared to those recorded by the colorimeter and portable spectrophotometer. Leaves stored at 0 and 10°C presented slight differences over time; however, in leaves at 20°C after 6 days, values significantly decreased. It was possible to obtain a 0.68 $R^2$ correlation between leaf chlorophyll content (mg/g FW) and the measurements recorded by the SPAD meter (Figure 7).

3.1.1.b Chlorophyll and carotenoids

In leaves stored at 0°C, chlorophyll concentration was higher after 6 days of storage (Figure 6). In leaves stored at 10°C, there were no significant differences over time. In leaves at 20°C, chlorophyll reached the lowest value after 6 days of storage.

Total carotenoid content followed a similar trend (Figure 6). In leaves stored at 0 and 10°C there were not differences over time. In leaves at 20°C, chlorophyll reached the lowest value after 6 days of storage.

3.1.2 Experiment 2: Intact and fresh-cut leaves at three maturity stages and three storage temperatures

Kale leaves cv. ‘Winterbor’ at three maturity stages were stored up to 29, 18 and 11 days at 0, 5 and 10°C, respectively (Figure 8).

3.1.2.a Respiration

Among different maturity stages, rates of respiration of intact and fresh-cut kale leaves cv. ‘Winterbor’ presented significant differences due to temperature (Figure 9), with higher
values in leaves stored at 10°C, followed by leaves at 5°C and finally with lower rates at 0°C. Among maturity stages, respiration rates at 5 and 10°C fresh-cut leaves were significantly higher than those of intact leaves, whereas at 0°C, no statistical difference was detected. There were differences due to storage time only in leaves stored at 5°C and 10°C, but no differences at 0°C. There were significant differences due to leaf maturity at all temperatures and formats (intact vs. processing). Immature leaves presented the highest respiration rates.

3.1.3 Experiment 3: Intact and fresh-cut leaves at two maturity stages

Kale leaves cv. ‘Winterbor’ were harvested at two maturity stages (Figure 10) and stored at 7.5°C for 12 days in intact and fresh-cut format (Figure 11).

3.1.3.a Marketability analysis

There was a greater decrease in overall visual quality (OVQ) in mature intact leaves than in immature leaves (Figure 12). Fresh-cut leaves followed the same trend, but with OVQ decreasing faster at both maturities and reaching lower scores at the last day of storage (day 12), particularly in pieces from mature leaves.

There was more yellowing in mature intact leaves than in immature leaves over time (Figure 12). Fresh-cut leaves followed the same trend, but immature leaves reached a higher yellowing score at the last day of storage.

There was a significant increase in decay/deterioration in mature intact leaves at day 12, while scores remained constant over time in immature leaves (Figure 12). Fresh-cut format followed a similar trend in mature leaves; however immature leaves showed increased decay scores at the last day of storage (day 12).
3.1.3.b  **Objective color**

In intact leaves, $L^*$ values slightly varied at both maturity stages. However in fresh-cut format at both maturities, $L^*$ values increased, especially after day 8 (Figure 13).

Hue angle values for immature intact leaves were lower initially and then were stable from day 4 to 12, an unexpected result. Hue values for mature leaves were similar over 8 days of storage but then decreased significantly by day 12, and were lower than Hue values for immature leaves. In fresh-cut leaves Hue decreased at both maturity stages after day 4. Hue angle values were the lowest for mature leaves on day 12 and were notably lower than values for immature leaves. The latter values were variable as indicated by the large standard error.

The color components $a^*$, $b^*$ and Chroma remained relatively constant between maturity stages over storage time (Table 2).

3.1.3.c  **Chlorophyll and carotenoids**

On day 0, total chlorophyll was the same for immature and mature leaves with a content of 1.27 and 1.34 mg/g FW, respectively (Table 3). Chlorophyll declined with storage time in leaves of both maturity stages (Figure 14). Although there was some variability, the content between the maturity stages was generally not different over the course of the experiment.

On days 0, 8 and 12 there were not significant differences between immature and mature leaves in terms of total carotenoid content (Table 3). Generally there were no differences with time under these experimental conditions as well (Figure 14).

3.1.3.d  **Antioxidant capacity**

On day 0, total antioxidant capacity of immature leaves, measured by FRAP method (3.08 mmole Fe$^{2+}$/100g FW) was significantly higher than that of mature leaves (2.52 mmole...
Fe$^{2+}$/100g FW) (Table 3). Antioxidant capacity of immature leaves was consistently higher than that of mature leaves throughout the experiment (Figure 15).

3.1.3.e Ammonia

There was an increase in ammonia concentrations over time for both maturity stages (Figure 16). However, concentrations in mature leaves were significantly higher than immature leaves at all evaluation times and ammonia concentrations increased first in mature leaves (Table 3).

Moreover, the ammonia content of immature increased by 3541% compared to day 0, vs. mature, which presented at increase of 7734% at the same conditions. Finally, total ammonia concentration of mature leaves was 155% higher than those at immature stage at the end of the storage.

3.1.4 Experiment 4: Intact and fresh-cut leaves at three maturity stages and two storage temperatures

Kale leaves cv. ‘Winterbor’ at three maturity stages were stored at 0 and 5°C up to 42 and 28 days, respectively, in intact (Figure 17) and fresh-cut (Figure 18 and Figure 19) format.

3.1.4.a Marketability analysis

There were similar trends in intact and fresh-cut leaves in terms of overall visual quality (OVQ). In intact format at 0°C (Figure 20, Table 4), the OVQ of immature, mature and overmature leaves decreased their OVQ by day 42 and overmature leaves reached the lowest scores (fair OVQ quality, product but not saleable). In fresh-cut format at 0°C (Figure 20, Table 4), pieces from leaves at all maturities reached lower scores than intact format at the last day of storage. This was particularly notable in the overmature leaves.
Leaves stored at 5°C showed similar trends. In intact format (Figure 22, Table 5), the OVQ of immature, mature and overmature leaves decreased over time, however overmature leaves reached the lowest scores at the last day of storage (day 28). Scores at day 28 were significantly lower than scores at the last day of storage of leaves at 0°C (day 42). In fresh-cut format (Figure 22, Table 5), all maturities reached lower scores than intact leaves at day 28, particularly mature leaves. At day 28, differences between immature and overmature leaves were non-significant.

For off-odors, there were similar trends in intact and fresh-cut leaves at 0°C (Figure 20, Table 4). In intact format, immature leaves did not present off-odors throughout the experiment, however mature and overmature leaves had an increase in off-odors score at the last day of storage (day 42). In fresh-cut format at 0°C, off-odors scores in immature leaves slightly increased at day 42. Immature and mature leaves stored at 5°C (Figure 22, Table 5) did not exhibit off-odors throughout the experiment. Conversely, off-odors scores in overmature leaves slightly increased at day 42.

Yellowing increased over time in intact and fresh-cut leaves stored at 0°C at all maturities, reaching moderately to moderately severe scores at day 42 (Figure 21, Table 4). Fresh-cut leaves stored at 0°C showed significantly higher yellowing scores than those intact. Leaves at three maturity stages stored at 5°C showed similar trends (Figure 23, Table 5), all of them exhibiting increasing yellowing over time, but immature leaves showed slightly lower yellowing scores. Scores at 5°C after 28 days were higher than leaves at 0°C after 42 days.

For decay and deterioration there were similar trends in intact and fresh-cut leaves stored at 0°C (Figure 21, Table 4). After 42 days, all maturities presented slight decay, but scores were higher in fresh-cut leaves, particularly in overmature stage. In intact and fresh-cut
format at 5°C, mature and overmature leaves had the highest decay/deterioration scores. Values were higher than intact leaves at 5°C and leaves stored at 0°C after 42 days.

In terms of discoloration and browning in intact and fresh-cut format at 0°C (Figure 21, Table 4), scores at all maturity stages slowly increased and overmature leaves obtained the highest scores at the last day of storage (day 42). In fresh-cut format at 0°C, scores were higher than in intact leaves. Intact leaves stored at 5°C (Figure 23, Table 5) showed a slow increase in discoloration/browning over time and overmature leaves had the highest scores at the last day of storage (day 28). In fresh-cut leaves at 5°C, there was a higher increase in scores at all maturities compared to intact leaves, but mature leaves had the highest scores at day 28.

3.1.4.b Chlorophyll and carotenoids

For intact and fresh-cut leaves at 0°C there were not significant differences in chlorophyll concentrations among maturity stages throughout the experiment (Figure 24). In intact leaves at 5°C, results were variable with no consistent trend in chlorophyll concentrations among leaves stages. In fresh-cut leaves stored at 5°C differences were more evident. However despite the variations, at the last day of storage (day 28) immature leaves exhibited the highest chlorophyll content, followed by mature and overmature leaves, which presented no significant differences between them.

In terms of total carotenoid content, there were not differences in terms of maturity stages, temperatures and processing over time (Figure 25).

3.1.4.c Ammonia

In intact leaves at 0°C (Figure 26, Table 6) ammonia content maintained relatively constant over 42 days. In fresh-cut leaves format at 0°C, overmature leaves exhibited higher ammonia concentration than immature and mature leaves after 42 days. In leaves at 5°C,
ammonia content increased from 21 days, particularly in mature and overmature leaves. In intact leaves after 28 days, overmature leaves presented a significant increase, followed by immature and mature leaves, with no differences between them. In fresh-cut leaves at 5°C after the same time period, overmature and mature leaves presented a notable increase in ammonia content, followed by immature leaves, which presented the lowest concentration.

3.1.4.d **Malondialdehyde (MDA)**

Initial MDA concentrations varied among stages of leaf maturity (Figure 27). In intact and fresh-cut leaves 0°C, although there were variations, MDA content was consistently and significantly higher in mature and overmature leaves and it remained relatively constant over time. In intact and fresh-cut leaves stored at 5°C, the trend was similar and mature and overmature leaves exhibited the highest MDA concentrations, with relatively constant values over time.

3.1.5 **Experiment 5: Fresh-cut leaves at three maturity stages and two storage temperatures**

Kale leaves cv. ‘Lacinato’ at three maturity stages (Figure 28) were fresh-cut processed and stored at 0 and 5°C for 42 and 28 days, respectively (Figure 29).

3.1.5.a **Marketability analysis**

In fresh-cut leaves stored at 0°C (Figure 30, Table 7), overall visual quality (OVQ) steadily decreased over time following the same pattern among maturities, however overmature leaves reached a slightly lower score at the last day of storage (day 42). At 5°C (Figure 31, Table 7), scores decreased constantly over time among maturities, however mature and overmature leaves reached the lowest scores at the last day of storage (day 28). At 5°C, OVQ final scores on day 28 were notably lower than final scores of leaves at 0°C on day 42.
For off-odors, scores in fresh-cut leaves stored at 0°C (Figure 30, Table 7) steadily increased over time following the same pattern among maturities, however mature leaves reached a slightly higher score at the last day of storage (day 42). In leaves at 5°C (Figure 31, Table 7), off-odors scores increased discretely at the three maturities, with no differences at day 28. In general, final scores at 5°C were similar to those at 0°C.

At 0°C, yellowing increased from day 14 until the last day of storage with no differences among maturities (Figure 30, Table 7). At 5°C (Figure 31, Table 7), yellowing increased at all maturities over time, but overmature leaves reached the highest scores at day 28. In general, final scores at 5°C were slightly higher than those at 0°C.

At 0°C, decay and deterioration were not observed until day 28 at all maturities (Figure 30, Table 7), however at day 42 it increased significantly, reaching the highest scores in mature and overmature leaves. In leaves at 5°C (Figure 31, Table 7) scores constantly increased at all maturities, however the highest yellowing score at day 28 was reached by mature and overmature leaves. In general, at 5°C final scores were higher than those at 0°C.

For discoloration and browning at 0°C (Figure 30, Table 7), scores started to increase from day 28 at all maturities, however at day 42 overmature leaves reached the highest scores. In leaves at 5°C (Figure 31, Table 7), yellowing increased in overmature leaves, which reached the highest scores at day 28. In general, final yellowing at 5°C was higher than in leaves stored at 0°C.

3.1.5.b **Objective color**

Changes in objective color were recorded by two instruments: colorimeter and portable spectrophotometer.
Colorimeter and spectrophotometer recorded small changes in L* values among maturities in leaves stored at 0°C: all maturities started with a similar value, then they decreased with time and finally increased at day 42 (Figure 32, Table 8, Table 9). At day 42 the highest value was reached by immature leaves, followed by mature and overmature leaves. In leaves stored at 5°C, colorimeter recorded slight changes in L* among maturities (Figure 33, Table 8): they started with a similar value, then it decreased with time and finally increased at the end of the experiment. The highest value was reached by mature leaves, followed by immature and overmature leaves. Values recorded by the spectrophotometer in leaves stored at 5°C (Figure 33, Table 9) were variable in the course of the experiment. The highest value at day 28 was reached by mature leaves, followed by immature and overmature leaves.

For the Hue angle values of leaves at 0°C (Figure 32, Table 8), colorimeter measurements showed a similar trend among maturities, with a decrease over time and finally a small increase at day 42. On the other hand, the spectrophotometer measurements showed a slight decrease with time in values among all maturity stages (Figure 32, Table 9). In leaves at 5°C (Figure 33, Table 8), the colorimeter Hue values showed a similar trend among maturities, with a decrease over storage time. The highest final value was observed in the immature leaves, followed by mature and overmature leaves, both with similar Hue values. In leaves at 5°C, the Hue values obtained by the spectrophotometer showed a constant decrease among maturity stages over time (Figure 33, Table 9), with immature leaves having the highest final Hue values.

In general, the trends in L* and Hue values were similar between instruments but absolute values recorded by the spectrophotometer were lower than those from the colorimeter. A linear regression was constructed and the level of R² correlation between L* and Hue angle values of the colorimeter and the portable spectrophotometer was 0.53 (Figure 34) and 0.72 (Figure 35), respectively.
For a*, b* and Chroma components, the colorimeter and portable spectrophotometer did not detect significant differences among maturity stages over storage time at 0 and 5°C (Table 8, Table 9).

3.1.5.c Chlorophyll and carotenoids

For total chlorophyll content at 0 and 5°C, there were no significant differences among immature, mature and overmature leaves throughout the experiment (Figure 36).

For total carotenoid content, at 0 and 5°C on day 0 (Figure 36), immature leaves had a significantly higher content than mature leaves; however from day 14 until the last day of storage, difference among maturity stages was non-significant.

3.1.5.d Ammonia

In general, in leaves stored at 0°C (Figure 37, Table 10), total ammonia content increased over time and it was significantly higher in overmature leaves throughout the experiment. In leaves stored at 5°C (Figure 37, Table 10), overmature leaves exhibited a notable increase from day 14. After 28 days of storage, mature and overmature leaves presented the same ammonia content, while the ammonia content of the immature leaves remained very low.

3.1.5.e Malondialdehyde (MDA)

In general, mature and overmature leaves had the highest MDA concentrations throughout the experiment, without significant differences between them. Their content remained relatively constant over storage time (Figure 38). The MDA content of immature leaves was consistently the lowest among maturities.
3.1.5.f  

Respiration

Among maturity stages, there were no significant differences due to time for fresh-cut ‘Lacinato’ leaves stored at 0 and 5°C. In leaves stored at 0°C (Figure 39), immature leaves had the highest respiration rates, followed by overmature leaves and mature leaves. In leaves stored at 5°C (Figure 39), immature leaves had the highest respiration rates, followed by mature leaves and overmature leaves. However, by comparing leaves at 0 and 5°C until day 24, immature leaves had the higher respiration rates, followed by mature and overmature leaves, with no significant differences between them. Until day 24, the respiration rates were significantly higher in leaves stored at 5°C.

3.2  Impact of Modified and Controlled Atmospheres on the Postharvest Performance of Kale Leaves

3.2.1  Experiment 6: Fresh-cut mature leaves stored in air or CA

Fresh-cut leaves cv. ‘Winterbor’ were stored at 5°C in three controlled atmospheres: Air (control); Air + 7.5% CO2 and Air + 15% CO2 (Figure 40).

3.2.1.a  Marketability analysis

There was a continual decrease in OVQ over time, with leaves stored in air (Control) having the lowest scores at the last day of storage (day 24), followed by atmosphere at 7.5% CO2 and finally by atmosphere at 15% CO2 (Figure 41, Table 11). Leaves stored in air reached an OVQ below the marketable limit at day 24. In the 7.5% and 15%CO2 atmospheres the final OVQ scores were slightly higher by day 24, but still below the limit of marketability. At 15% CO2 final scores were higher than the other treatments, reaching a fair OVQ.

For off-odors, there was an increase from day 10 in 15% CO2, which presented the highest scores (moderately severe) at the last day of storage (day 24) (Figure 41, Table 11). In
the control there was absence of off-odors throughout the experiment. At 7.5% CO2 off-odors scored were slight to moderate at day 24.

There was a constant increase in yelIowIlIg over time among different atmospheres, however the highest scores at the last day of storage (day 24) were reached by the control, with yellowing scores between moderately severe and severe. Treatment at 15% CO2 had slightly lower yellowing scores than leaves stored at 7.5% CO2 (Figure 41, Table 11).

For decay and deterioration, increases in scores were evident only after 20 days in the control, which reached the highest scores at the last day of storage (moderately severe) (Figure 41, Table 11). In atmospheres 7.5 and 15%CO2 it was detectable at day 24, reaching scores of “moderate” and “slight”, respectively.

There was a constant increase in discoloration and browning over time, starting after day 10, with control leaves having the highest scores at the last day of storage (moderately severe). At 7.5 and 15% CO2 atmospheres, final values were lower than the control (moderate discoloration/browning), and did not present differences between them (Figure 41, Table 11).

3.2.1.b Chlorophyll and carotenoids

All treatments followed the same trend, exhibiting a constant decrease, with days 20 and 24 showing the lowest chlorophyll content (Table 12). At the last day of storage (day 24), leaves at 7.5% and 15% CO2 atmospheres had the highest chlorophyll concentration, followed by the control, which exhibited the lowest content (Figure 42).

For total carotenoid content (Figure 42, Table 12), no differences were observed throughout the experiment at different atmospheres.
According to the general linear regression that was constructed for the complete study (Experiments 1, 3, 4, 5 and 6), the level of $R^2$ correlation of total chlorophyll content to total carotenoid content was 0.92 (Figure 48).

3.2.1.c  
**Ammonia**

Total ammonia content significantly increased over time in the CA treatments (Figure 43, Table 12). The 15%CO2 atmosphere reached the highest ammonia concentration after 24 days, followed by 7.5% CO2 and finally control, in which a low concentration was maintained.

3.2.1.d  
**Malondialdehyde (MDA)**

In leaves stored in air (Control) and 15% CO2, there were not significant differences in MDA concentration over storage time. In leaves at 7.5% CO2, there were slight differences over time (Table 12). At the last day of storage (day 24), control leaves had the highest MDA content and there were no differences between concentrations in leaves stored under 7.5 and 15% CO2 treatments (Figure 44).

3.2.2  
**Experiment 7: Fresh-cut leaves stored in MAP at three temperatures**

Fresh-cut kale leaves cv. ‘Winterbor’ packaged in microperforated bags obtained from a commercial fresh-cut processor and stored at 0, 5 and 10°C for 20, 20 and 16 days, respectively (Figure 45).

3.2.2.a  
**Marketability analysis**

There were clear differences in overall visual quality (OVQ) of fresh- cut kale leaves in relation to storage temperature (Figure 46, Table 13). The cut leaves stored at 0°C up to 20 days presented the highest OVQ scores over time, followed by cut leaves at 5 and 10°C stored for 20 and 16 days, respectively.
Leaves stored at 0°C presented the lowest off-odor scores over time, reaching a score of “slight” at day 20 of storage, followed by leaves at 5°C that reached moderately severe off-odors (Figure 46, Table 13). Leaves at 10°C stored for 16 days reached the highest off-odors scores, reaching a score of 5 (severe) at day 16.

For yellowing, leaves stored at 0°C up to 20 days had the lowest scores over time (Figure 46, Table 13), followed by leaves at 5°C and finally leaves at 10°C, which exhibited the highest yellowing scores.

For decay and deterioration (Figure 46, Table 13), leaves stored at 0°C up to 20 days presented the lowers scores over time, followed by leaves at 5°C. Leaves at 10°C stored for 16 days reached the most severe scores, becoming unusable.

Fresh-cut leaves stored at 0°C up to 20 days presented the lowest scores of discoloration and browning over time, followed by leaves at 5°C (Figure 46, Table 13). Leaves at 10°C stored for 16 days reached the most severe scores, becoming unusable.

3.2.2.b Gas analysis

The O2 concentrations in bags stored at 0 and 5°C were relatively constant compared to bags stored at 10°C. In the latter, O2 concentration significantly decreased during day 12 storage period (Figure 47).

As expected for microperforated packaging, the CO2 concentration was significantly higher in bags stored at 10°C than in bags at 0 and 5°C. On the last day of storage, CO2 concentrations were higher (64%) than at the beginning of the storage. On the other hand, CO2 concentration in bags at 5 and 0°C remained relatively constant over time.
4. DISCUSSION

4.1 Impact of Processing, Leaf maturity and Temperature on the Postharvest Performance of Kale Leaves

The impact of leaf maturity on the postharvest performance of kale leaves, and particularly marketable attributes, was influenced by the cultivar, temperature and fresh-cut processing. In other words, the increasing level of deterioration over time in leaves at a more advanced age was enhanced by fresh-cut processing and increasing storage temperatures, particularly in cv. ‘Winterbor’. Conversely, in cv. ‘Lacinato’, leaf maturity and processing did not have a significant impact on the postharvest performance at low temperatures (0 and 5°C). Cultivar ‘Lacinato’ was considerably more stable and presented better postharvest performance than cv. ‘Winterbor’. A major difference between cultivars is the greater homogeneity of leaf morphology of cv. ‘Lacinato’, which exhibits a uniform color on the surface, suggesting the same for chlorophyll and carotenoid distribution. Conversely, leaves of cv. ‘Winterbor’ are more heterogeneous even when from apparently the same stage of maturity. They exhibited a mosaic of green colors in different shades and intensities, which could be associated to an unequal distribution of chlorophyll and carotenoids among the leaf tissue. This evidence is suggesting clear differences due to genotype in kale, which is supported by studies from Zietz et al. (2010), Mercadante & Rodriguez-Amaya (1991) and Balkaya & Yanmaz (2005).

Overall, immature leaves had the best marketability scores, particularly at the last days of storage. Overmature leaves exhibited the worst postharvest performance, followed by mature leaves, which reached the highest scores in some defect attributes. Differences due to leaf age were accentuated at temperatures higher than 0°C and also with fresh-cut processing. Unfortunately, interaction among these three factors has been poorly studied. Most of the
literature addresses the impact of separate factors on the postharvest performance of leafy greens.

Couture et al. (1993), reported a better postharvest quality in immature lettuce leaves in minimally processed format, compared to mature and overmature leaves, all stored during 4 days at 2.5°C, followed by transfer to air at 20°C for 1 day. Similarly, Chiesa et al. (2003) observed that fresh-cut lettuce leaves harvested at 45 days (“intermediate maturity”) performed significantly better in terms of overall visual quality compared to leaves harvested after 30 and 60 days. Moreover, Koukounaras et al. (2007) reported a significant increase in yellowing scores after 10 days of storage at 10°C in young, fully expanded and mature intact rocket (Eruca sativa Mill.) leaves. Among them, mature leaves (older) had slightly higher yellowing scores, followed by fully expanded (intermediate) and young leaves, which had the best scores. Better marketable attributes (overall visual quality and cut-edge browning) were observed by Gil et al. (2012) in less mature fresh-cut romaine lettuce stored in active modified atmosphere packaging (MAP) for 3 days at 4°C plus 9 days at 7°C.

Furthermore, Mateos et al. (1993) reported between 0-10% (low score) of senescent browning in intact lettuce heads (cv. ‘Vanguard’) after storage in air at 2.5°C for 10 days, 20 days; or 20 days followed by transfer to air at 20°C for 12 h. Conversely, minimally processed midrib stored in air showed low to high senescent browning scores (30 to 90% surface affected) under the same conditions. Green tissue exhibited low to moderate senescent browning scores (20 to 40% surface affected) as well.

Among the critical attributes of fruit and vegetable quality are color and appearance, flavor (taste and aroma), texture and nutritional value (Barrett et al., 2010). This was critical on leaves stored at temperatures ≥5°C, opposite to those at 0°C in this study, which exhibited marginal changes in marketability attributes. The dramatic loss of quality in storage at 10°C
could be explained by the use of fresh-cut processed format and commercial material. Processing significantly increases the rates of respiration, biochemical changes, microbial degradation, which leads to a faster physiological deterioration (Brecht, 1995; Garcia & Barrett, 2002; Rico et al., 2007; Francis et al., 2012) and summed to the use of high temperatures the mentioned processes are enhanced.

Consequently, it is critical to consider the interaction among these factors, because they will influence the appearance of the product, which is one of the most critical quality attributes, and in consequence will determine its acceptance or rejection by consumers (Barrett et al., 2010; Kramer, 1965).

Compositional analyses were able to provide more accurate information about the metabolic status of the leaf tissue and are complementary to the objective color measurements (discussed later) and marketability analyses.

Total chlorophyll content was higher in immature and mature leaves than overmature leaves from cv. ‘Wintebor’ at harvest (day 0). This initial difference determined the postharvest performance among maturity stages, particularly at temperatures higher than 0°C and in presence of fresh-cut processing. For this cultivar, immature and mature leaves were harvested at two different developmental stages that had not reached full expansion but that had reached their highest chlorophyll level. According to Bohmert et al. (1998) and Williams (2012), leaf development is initiated with the formation of leaf primordia at the flanks of a multicellular tissue located in the shoot apex, called Shoot Apical Meristem (SAM). This active process of cell division is followed by the lateral expansion of the margins of a leaf primordium (Poethig, 1997). After completion of expansion, the phase of leaf maturity starts and extents until the appearance of the first senescence symptom, represented by chloroplast breakdown (Guiboileau et al., 2010).
The initial difference among maturity stages in terms of total chlorophyll content in intact and fresh-cut leaves from cv. 'Winterbor' stored at 0°C disappeared over time, showing no variability at the last day of storage, which is consequent with the reduced metabolic activity of commodities stored at 0°C (Kader, 2002a) and the minimization on chlorophyll changes. However in leaves that were stored at temperatures higher than 0°C, particularly in fresh-cut format, the difference between maturity stages became evident from day 14, reaching its higher level at the end of the storage (day 28), where immature leaves exhibited the highest chlorophyll concentrations. In other words, leaf maturity showed a significant effect on total chlorophyll content depending on the use of increased temperatures (>0°C) and fresh-cut processing. Unfortunately, the combination of these three factors (temperature, fresh-cut processing and leaf maturity) in postharvest and their influence on the compositional content of leafy vegetables has not been well studied.

Nonetheless, Page et al. (2001) observed a significant and rapid decrease in chlorophyll content in broccoli florets stored at room temperature when compared to those stored at 4°C. Hodges & Forney (2001) reported a significant decrease (75%) in total chlorophyll content of minimally processed spinach leaves after 16 days of storage at 10°C. Likewise, Xu et al. (2012) observed losses in total chlorophyll in broccoli florets after 1 day of storage at 15°C. Carnelossi et al. (2002) reported that fresh-cut kale leaves stored in polyolefin multilayer packaging of high permeability or polyethylene terephthalate (PET) boxes at 1°C, both chlorophyll and carotenoid content remained constant during the 15 days of storage. However, leaves stored at 10°C exhibited a significant decrease in both parameters independent of the type of packaging.

Chlorophyll degradation and losses caused by temperature, fresh-cut processing and leaf maturity were consistent with an increased leaf yellowing, as seen in terms of marketable attributes. A series of catabolic processed are triggered with temperature increase, including ethylene production, protein degradation, chlorophyll breakdown, membrane and lipid
peroxidation (Brecht, 1995; Wills et al., 1999). Moreover, reactive oxygen species and lipid peroxidation generated as a consequence of tissue wounding by fresh-cut processing can lead to chlorophyll degradation (Rolle & Chism, 1987; Zhuang et al., 1997; Reyes et al., 2007).

The effect of maturity in kale leaves from cv. ‘Lacinato’ was substantially different compared to the results obtained in cv. ‘Winterbor’. Total chlorophyll content generally did not differ among maturity stages over time at 0 and 5°C, even with fresh-cut processing. Only at the last day of storage at 5°C, there was a significant decrease in chlorophyll concentration, which was consistent with marketability evaluations. This behavior could be partially explained by the fact that leaves of cv. ‘Lacinato’ were highly homogeneous compared to those of cv. ‘Winterbor’. Also, the use of low temperatures limited the breakdown of chlorophyll, even with minimally processing. Among leafy greens, these observations were similar to those found by Ferrante et al. (2008), who reported no consistent differences between whole and cut Swiss chard chlorophyll concentrations of leaves after 12 days stored at 4-5°C under light or dark conditions.

On the other hand, total carotenoid content in both cultivars was not affected by leaf maturity and remained relatively constant over time even at temperatures higher than 0°C and after fresh-cut processing. These results were not consistent with Lesfrud et al. (2007), who reported the maximum carotenoid accumulation occurring in mature fully expanded kale leaves cv. ‘Winterbor’. de Azevedo & Rodriguez-Amaya (2005) reported no significant differences between β-carotene, lutein, violaxanthin and neoxanthin contents between young and mature kale leaves cv. ‘Manteiga’ from organic production, but significant differences in leaves from conventional production.

Consistent to this study, Ferrante et al. (2008) observed no differences in total carotenoid content of Swiss chard (Beta vulgaris L.) stored at 4-5°C for 12 days in both intact and fresh-cut format. Moreover, Ferrante et al. (2009) observed no significant changes between
intact and fresh-cut lamb’s lettuce (Valerianella olitoria) leaves in terms of total carotenoid content during storage 4°C for 8 days. Kobori et al. (2011) also reported no significant differences in carotenoids neoxanthin and violaxanthin, but a decrease in lutein and β-carotene in fresh-cut kale leaves stored at 1°C in the dark for 15 days. On the other hand, at 11°C in the dark all the carotenoids decreased after 5 days of storage.

Additionally, de Acevedo & Rodriguez-Amaya (2005) observed a significant decrease in β-carotene, violaxanthin and neoxanthin in fresh-cut kale leaves after one day of storage at approx. 7-9°C, after which values remained constant until the fifth day of storage.

The absence of statistical changes in total carotenoid content in the present study could be explained by the presence of a masking effect of a fraction of carotenoids that remained relatively constant, over other carotenoids that could have decreased during the course of the experiment. Furthermore, the use of temperatures lower than 10°C in this study may have limited the degradation of carotenoids, which suggests that more abusive temperatures would negatively affect the concentration of these pigments.

Consequently, visible changes leading to yellowing are mostly associated to chlorophyll breakdown and subsequent exposure of carotenoid pigments. According to Korus (2012b), kale leaves are considered to be a rich source of carotenoid compounds, which are masked by green chlorophyll.

In general, values of total chlorophyll and carotenoid contents in this study were consistent with previous reports (Humphries & Khachik, 2003; Kopsell et al., 2007; Yahia & Ornelas-Paz, 2010; Sikora & Bodziarczyk, 2012) and only slightly higher than observations made by Khachik et al. (1986). Their level of correlation in this study was also high, which was consistent with their relative proportional behavior in postharvest under the mentioned variables (fresh-cut processing, leaf maturity and temperature). Chlorophyll and carotenoids are the main
pigments. On the one hand, chlorophyll allows the absorption of light mostly in the blue portion of the electromagnetic spectrum. On the other hand, carotenoids serve key physicochemical and photophysical functions, which are associated to structural roles in the organization of photosynthetic membranes, participation in light harvesting, energy transfer, quenching of chlorophyll excited states and single oxygen, and interception of deleterious free oxygen and organic radicals as well (Gitelson et al., 2002).

Total antioxidant capacity was directly affected by leaf maturity. Immature leaves had significantly higher antioxidant activity than mature leaves. Antioxidant activity of leaves at both ages remained relatively constant during storage at 7.5°C. These results were consistent to those of Nantitanon et al. (2010), who reported a higher phenolic content, free radical scavenging activity and reducing power in young leaves of *Psidium guajava*, compared to middle and old age leaves. Moreover, Wang & Lin (2000) found higher ORAC values and total phenolic content in young leaves from different berry crops, compared to older leaves. Korus (2011) observed a higher polyphenol content and antioxidant activity in kale leaves harvested 18 weeks after planting of seedlings, compared to younger leaves (harvested at 10 and 14 weeks). On the other hand, similar results were observed by Hodges & Forney (2001) in spinach leaves cv. ‘Spokane’, which exhibited a decrease in total ascorbate content only after 12 days of storage at 10°C, and no significant difference in total glutathione content after the same period. This indicates that antioxidant content (expressed in these two compounds) remained stable for relatively long storage time even under exposure to temperatures that can be considered abusive. However, further studies are required to determine the evolution of antioxidant content at a broader range of different development stages in kale leaves.

Total ammonia content was significantly affected by a more advanced leaf maturity at harvest. Overmature leaves exhibited the highest ammonia concentrations, followed by mature and immature leaves. This difference was enhanced by the influence of fresh-cut processing.
and increased storage temperatures. Interestingly, this was coincident with higher off-odors scores, also consistent with the observations of Tudela et al. (2013) in baby spinach, thus indicating that ammonia may be a useful indicator of stress as proposed by Cantwell et al. (2010). However, this was not the case because yellowing preceded increases in ammonia concentration. Currently there are not reports about the influence of organ age or maturity at harvest on total ammonia production. Available information about ammonia content in postharvest include observations about differences among sections (floral, middle and base) of broccoli branchlets from King & Morris (1994) and between top and bottom portions of asparagus spears (Enriquez et al, 2001).

Large amounts of ammonia can be endogenously generated when proteins are degraded (Givan, 1979; King et al., 1990), which is a well-documented postharvest phenomenon when the onset of senescence is near or has begun. At high concentrations ammonia is toxic to plant cells, but within the plant it is normally assimilated by the enzyme glutamine synthetase. However the decrease in its activity in postharvest is a consequence of the global metabolic decline or catabolism that characterizes this period (Enriquez et al., 2001). Temperatures higher than 0°C, in combination with fresh-cut leaf material, resulted in differences in ammonia content among maturity stages and triggered its dramatic increase. Considering that fresh-cut processing in combination with temperature promote faster physiological deterioration and biochemical changes (Garcia & Barrett, 2002; Kader, 2002a; Rico et al., 2007), the dramatic increase in ammonia production in kale leaves can be explained as the result of the interaction and synergy between both factors. Moreover, overmature leaves showed symptoms of the senescence syndrome at harvest; therefore the catabolic processes leading to protein degradation were enhanced by temperature increase and fresh-cut processing.
There are few studies about the impact of ammonia production and fresh-cut processing in vegetables, while the effects of temperature abuse have been well documented. Matsui et al. (2004) reported a significant increase in ammonia content in moso bamboo shoot stored at 20°C for 9 days, compared to those stored at 1°C. A similar trend was observed by Baclayon & Matsui (2008), with a considerable increase in ammonia concentration in broccoli florets stored at 20°C for 5 days, compared to florets stored at 5°C. Furthermore, an increase in ammonia concentration was found in asparagus spears after 5 days of storage at 25°C (Bhowmik et al., 2008). Similarly, a significant and rapid decrease in total protein content was reported in broccoli florets stored at room temperature when compared to those stored at 4°C (Page et al., 2001).

Total malondialdehyde (MDA) content was positively influenced by leaf maturity. Overall, mature and overmature leaves had higher MDA concentrations than immature leaves at harvest (day 0) and this difference remained constant over time until the end of the storage. These results were consistent with the observations of Dhindsa et al. (1981) in tobacco leaves, where a rapid increase in membrane permeability and lipid peroxidation (expressed in MDA content) was found after full leaf expansion was reached. This point was coincident with a decrease in total chlorophyll and total protein content.

Processing and temperature did not have any significant effect on the total MDA content. This was interesting, considering that MDA is an end product of lipid peroxidation that has been traditionally used as an indicator of membrane injury (Yuan et al, 2010), hence as fresh-cut processing is a dramatic representation of tissue wounding, it would be consistent to infer that fresh-cut leaves may present a significantly higher level of MDA. This however was not the case, which can be explained by the use of relatively low temperatures during storage (0 and 5°C). Zhuang et al. (1997) observed a 10-fold increase in lipid peroxidation derivatives (MDA and thiobarbituric acid-reactive monoaldehydes) in broccoli buds stored at 23°C for 144 h, compared to no changes at 2°C. Moreover, Balouchi et al. (2011) reported a significant increase
in MDA production in florets of 5 different broccoli cultivars stored at 0°C for 40 days, compared to those stored at 20°C for 3 days. In other words, the use of low temperatures could have limited the lipid peroxidation generated by fresh-cut processing, however this range of temperatures were able to provide a reliable representation of real conditions in market. We suggest that temperatures higher than 5°C would be able to trigger lipid peroxidation in fresh-cut tissue.

Compositional changes were directly correlated with respiration rates, which are an indicator of the metabolic status of a commodity. Respiration was significantly influenced by leaf maturity in kale leaves. Despite some variations between mature and overmature leaves, immature leaves presented significantly higher respiration rates among temperature and between intact and minimally processed leaves. Vegetative tissue, such as leaves and stems, or floral meristems exhibits higher respiration rates than mature fruits and dormant organs. From a physiological perspective they are still developing and in consequence synthesizing new material, thus demanding energy to maintain the commodity’s living status. Tissues at an earlier developmental stage also present higher respiration rates compared to mature organs (Kader, 2002a; Saltveit, 2004). Furthermore, the stage of leaf maturity in which leafy vegetables are harvested is crucial for those that are destined for fresh-cut market, because the rate of deterioration related to respiration rates and metabolic (catabolic) processes are usually higher in younger developing leaves (Gil et al., 2012).

Similarly, processing significantly accelerated respiration rates in this study. This was consistent with the observations of Kim et al. (2004), who reported respiration rates of 20 and 13 μL g⁻¹ h⁻¹ at 5°C after 24 h in intact leaves of white and violet savoy (colored varieties of kale), respectively. Conversely, values of 28 and 26 μL g⁻¹ h⁻¹ were observed in fresh-cut leaves of white and violet savoy, respectively, which represents about a 30% increase. Cantwell & Suslow (2002) reported similar values for intact kale, classifying it as a horticultural commodity with a
“very high” respiration rate. On the other hand, Carnelossi et al. (2005) reported respiration rates around 5 μL CO₂ g⁻¹ h⁻¹ in minimally processed kale leaves stored at 1, 5 and 10°C, measured 12 h after harvest, however these values were significantly lower than in the present study. Fresh-cut processing implies the wounding and rupture of leaf tissues. As a consequence, chemical and metabolic reactions are initiated and will short the storage life, which include increased respiration rates (Bolin & Huxsoll, 1991; Brecht, 1995).

Likewise, temperature strongly affected the rates of respiration. Temperature is considered the key environmental factor that most influences the deterioration rate of harvested plant organs because it increases the rate at which metabolic reactions occur. Moreover, for each increase of 10°C above the optimum temperature, which in leafy greens is 0°C, the rate of deterioration increases by two to three-fold (Kader, 2002a). These results were also consistent with Carnelossi et al. (2005), who reported an increase in the respiratory rate and ethylene production of minimally processed kale leaves after 4 hours of storage at 25°C.

Depending on the type of commodity, the duration of the storage and other considerations, there is variability regarding the exact threshold under a temperature can be considered abusive in postharvest storage. Cantwell & Suslow (2002) indicated that storage temperatures for fresh-cut products must be between 0 and 5°C. Higher values can be considered temperature abuse, which significantly increases quality losses and accelerates spoilage. Sela & Fallik (2009) consider that values above the refrigeration temperature (4 to 5°C for domestic refrigerators) are abusive and promote microbial growth and proliferation in storage. On the other hand, Whitaker (2002) considers that temperature abuse is holding the product at 10°C or above.

Additionally, the use of commercial plant material may have aggravated the temperature effects, because commercial handling from farm to consumer often involves inappropriate
manipulation during harvest, cooling, transportation and marketing that increases the rate of
damage and wounding that has been already exerted by fresh-cut processing (Thompson,
2002; Thompson & Crisosto, 2002; Bollen, 2006; Lurie, 2009).

In conclusion, the interaction between these three factors: leaf maturity at harvest
(immature vegetative tissue), temperatures higher than 0°C and fresh-cut processing
(wounding) is suggesting to be synergistic and result in hastening of the deterioration process,
as observed in the present study.

4.2 Impact of Modified and Controlled Atmospheres on the Postharvest Performance
of Kale Leaves

The composition of the air in the atmosphere is: 78.08% Nitrogen (N2), 20.95% Oxygen
(O2) (both corresponding to the major gases); 0.93% Argon (Ar), 0.03% Carbon Dioxide (CO2),
among others (minor gases) (Williams, 2004). However, the gases of interest in postharvest
physiology are oxygen, carbon dioxide and water vapor. In consequence, the concept of
Controlled Atmospheres (CA) implies the modification, by removal or addition, of the gases
surrounding the commodity, commonly involving the reduction of oxygen and/or elevation of
carbon dioxide concentrations. The application of CA should be considered a supplement to
temperature and relative humidity management (Kader, 2002b). Among the benefits of the
application of CA it is possible to mention the retardation of the senescence process, slowing of
respiration and decrease of decay incidence and severity (Kader, 2002b).

Marketability attributes were significantly affected by storage of kale leaves in air + 7.5%
or 15% CO2 compared to storage in air at 5°C. Overall, the best results at the end of storage
(day 24) were reached by Air + 15% CO2 in terms of overall visual quality (OVQ) and
decay/deterioration. Both CO2 atmospheres showed reduced yellowing and
discoloration/browning. On the negative side, Air + 15% CO2 treatment also reached the
highest off-odors scores. Among the potential harmful effects of controlled atmospheres is the development of off-odors at very low O2 or very high CO2 concentration due to fermentative metabolism or anaerobic respiration (Kader, 2002b). Moreover, Fonseca et al. (2001) reported the presence of off-odors that were below the level of acceptability in fresh-cut kale in a controlled atmosphere of Air + 20% CO2 after 4 days of storage at 20°C, even when other compositional parameters were not negatively affected.

The maximum CO2 concentration tolerated by kale is 15% (Kader, 2002b), which was consistent with the OVQ and decay/deterioration scores in this study. Assuming that recommendation is based on storage at 0°C, the Air + 15% CO2 treatment at 5°C could be considered abusive or beyond the tolerance level for kale in the fresh-cut format. The tolerance of kale to carbon dioxide decreases with a reduction in O2 level, and the tolerance to reduced O2 also increase with an increase in CO2 level (Kader, 2002b).

Fresh-cut commodities exhibit higher respiration rates and in fresh-cut kale, rates were doubled the rate of intact leaves (Cantwell & Suslow, 2002), which was consistent with the observations made in this study. In consequence, temperature control and management is more restrictive (Cantwell & Suslow, 2002; Garcia & Barrett, 2002; Barrett et al., 2010) because an apparently small increase in temperature from 0 to 5°C can result in considerable changes in postharvest performance.

Total chlorophyll content was significantly affected by the CO2 atmospheres compared to air-stored kale. Both treatments retarded the loss of chlorophyll, which remained relatively constant and without significant variations during 24 days of storage. These results were consistent with those of Fonseca et al. (2001), who described retention of total chlorophyll content and objective color with atmospheres up to 15 and 20% CO2 in air.
Moretti et al. (2003) reported decreased chlorophyll losses in fresh-cut kale leaves of cv. ‘Manteiga’ stored at 5°C under a controlled atmosphere of 3% CO2 + 4% CO2 for 6 days, compared to 5% CO2 + 5% CO2 and a control.

Conversely, total carotenoid content remained relatively constant in air stored. Likely, the low temperature (5°C) prevented the breakdown of the carotenoid pigments, even in fresh-cut kale. In the course of this study, these compounds were shown to be highly resistant to degradation under temperatures below 10°C.

Total ammonia content was significantly and negatively affected by higher concentrations of CO2. The dramatic increase in ammonia production in the 15% CO2 atmosphere is consistent with the observations of Cantwell et al. (2010), who reported increases in ammonia, tissue discoloration and increases in fermentative volatiles in fresh-cut spinach leaves stored in an atmosphere of Air + 15% CO2. Moreover, Tudela et al. (2013) reported that increasing CO2 concentration significantly increased tissue damage with ammonia release and decreased protein content. Hence, this is indicating that ammonia could be a useful indicator of stress in commodities stored in inappropriate controlled or modified atmospheres (Cantwell et al., 2010), because based in our observations, there is a significant difference between visual and subjective observations and what is occurring internally at a tissue, cellular and molecular level in the commodity. In other words, marketability evaluations do not provide a completely accurate assessment of postharvest performance and need to be complemented with compositional analyses. For this leafy vegetable, changes in the concentration of ammonia appear to provide a sensitive indicator of the changes associated with the senescence and deteriorative processes.

Malondialdehyde concentrations were barely affected by the CO2 treatments and did not exhibit significant differences with respect to the control. Again, the use of a low temperature
(5°C) prevented lipid peroxidation in kale leaves; however this range of temperatures provided a real representation of the market and industry conditions. Further studies with the use of higher storage temperatures may be useful to determine the impact of the atmosphere treatments on MDA concentrations.

Based on marketability and composition analyses; we recommend the use of Air + 7.5% CO2 could be recommended for kale. Nonetheless, this is preliminary data and requires other compositional analyses, such as antioxidants and glucosinolates to confirm this conclusion.

In terms of modified atmosphere packaging (MAP), temperature had a significant impact on the concentration of oxygen and carbon dioxide in the bags. As expected, carbon dioxide concentrations increased as temperature increased to about 5, 8 and 12% after 10 days at 0, 5 or 10°C, respectively. On the other hand, oxygen concentrations decreased as storage temperature increased with averages of 16, 14, and 9%, respectively. These results were consistent with Kobori et al (2011), who observed significant increases in CO2, which were inversely proportional to O2 concentrations (decreasing over time) in minimally processed kale leaves after 17 days stored in air at 1°C in dark, or 11°C in dark or light. As temperature increased, respiration rates increased causing more CO2 accumulation while oxygen was depleted (Saltveit, 2004). Kale leaves are among the horticultural commodities possessing a “very high” respiration rate (between 20-30 mL CO2/kg-hr) (Kader, 2002a) and wounding caused by fresh-cut processing enhanced respiration (Cantwell & Suslow, 2002).

Consistent with this, Fonseca et al. (2001) observed an increase in off-odors scores in fresh-cut kale leaves stored in 20% CO2 + 21% O2 and 2% O2 (no CO2) atmospheres after 4 days at 20°C. At 2% O2 + 20% CO2 atmosphere, off-odors scores crossed the limit of acceptability after 5 days at 20°C. Conversely, changes in objective color were not significant in low oxygen atmospheres and no carbon dioxide under the same conditions. Kobori et al (2011),
studied fresh-cut kale leaves after 17 days stored in air at 1°C in dark, or 11°C in dark or light. In this study, sensory quality defects (discoloration, wilting, senescence and undesirable odors) changed significantly in all treatments, however leaves at 11°C reached the highest scores and had the lowest overall appearance. This is suggesting that in addition to temperature, changes in marketable quality can be explained by the high oxygen levels present in the packages at the beginning of our experiment (15%). Considering that kale is a commodity having high respiration rates and also the use of fresh-cut material can explain the significant changes in marketable quality under these conditions.

4.3 Analysis of Color and Chlorophyll Estimates

Objective color measurements were made using two instruments: colorimeter and portable spectrophotometer, which differ in their level of accuracy (portable spectrophotometer is more precise). Here, objective color exhibited a clear pattern associated to loss of green (increasing L* value) and yellowing (decreasing Hue angle value) over time particularly in kale leaves stored at temperatures higher than 5°C. These results correspond to decreasing SPAD values and decreasing concentrations of chlorophyll and carotenoids as well. This was also consistent with Amarante et al. (2008), who reported that intense green leaves would exhibit Hue angle measurements close to 180° and L values between 0 and 50 (darker); while leaves presenting yellowing had a Hue angle close to 90° and L values between 51-100 (lighter).

Overall, leaf maturity did not influence objective color values consistently over time. Although it was possible to observe differences associated to maturity at day 0, due the heterogeneity of the results during storage, particularly in cv. ‘Winterbor’, it was not possible to make general conclusions. In cv. ‘Lacinato’, leaf maturity did not affect objective color throughout storage.
Results from both color measurement instruments followed the same trend, but differed in absolute values. The colorimeter registered higher values compared to the portable spectrophotometer, although both instruments were calibrated with a standard white tile. Nonetheless, the strong positive linear relationships in terms of L* and Hue angle value suggest that both instruments are able to provide a reliable non-destructive assessment in terms of color. However, destructive compositional analyses are not replaceable yet and constitute the most accurate indicator of the pigment status of a commodity.

Additionally, more accentuated trends related to loss of green were observed in cv. ‘Winterbor’ than in cv. ‘Lacinato’. Cultivar ‘Lacinato’ has a uniform dark green color on the surface, suggesting the same for chlorophyll and carotenoid distribution. Conversely, leaves of cv. ‘Winterbor’ are more heterogeneous in terms of color and leaf morphology.

Another non-destructive method for estimating chlorophyll content was used in this study. The SPAD Chlorophyll-meter showed that SPAD values decreased as storage temperature increased, which was consistent with losses in chlorophyll content. These results were consistent with Amarante et al. (2008), who reported values similar to our study (range between 18-46 SPAD units) in kale leaves that were harvested at different ages, thus exhibiting a range of colors from yellowish green to dark green.

Nonetheless, the level of correlation between chlorophyll concentration and SPAD meter values differed depending of the cultivar. Cultivar ‘Lacinato’ exhibited a higher linear correlation than cv. ‘Winterbor’, which could be explained by its uniform color on the surface. Conversely, the heterogeneity of cv. ‘Winterbor’ increases the variability of the color readings and decreases the level of correlation to total extracted chlorophyll as well. Despite this differences, the use if this device still constitutes a reasonably reliable method for non-destructive estimation of total chlorophyll. Observations made by Markwell et al. (1995) found correlations around 0.95 for
soybean and maize leaves. Furthermore, Monje & Bugbee (1992) reported a correlation of 0.93 for wheat, rice and soybean leaves. However kale leaves, particularly cv. ‘Winterbor’, are morphologically different (higher thickness, curly edges and fewer smooth surfaces), which can lead to lower correlation coefficients.
5. CONCLUSIONS

Leaf maturity at harvest had a significant impact on the postharvest performance of kale leaves in combination with fresh-cut processing and storage temperatures higher than 0°C. In terms of marketable quality, immature leaves performed better than mature and overmature leaves which exhibited higher rates of deterioration. Fresh-cut processing and increased storage temperatures accelerated the overall rates of deterioration and leaf senescence.

Regarding chemical composition, immature leaves had a higher total antioxidant capacity compared to mature leaves. Total ammonia, associated with protein degradation under stress conditions or proximity to the onset of senescence, was influenced by leaf maturity, processing and temperature. Mature and overmature leaves had a dramatic increase in ammonia concentrations over time, in contrast to small changes in immature leaves. Fresh-cut processing, which is associated to tissue wounding, and temperatures higher than 0°C also resulted in increased contents of total ammonia. Immature leaves had lower total malondialdehyde (MDA) content than mature or overmature leaves, which were not affected by processing or storage temperature. MDA concentrations are indicators of the rate of lipid peroxidation and membrane breakdown in postharvest and under stress conditions. Total chlorophyll and carotenoid contents were slightly affected by leaf maturity and processing in temperatures below 5°C.

Respiration rates were highest in immature leaves, which was consistent with their condition of vegetative tissues in active development, followed by mature and overmature leaves, which exhibited the lowest rates. Respiration was also increased by fresh-cut processing and storage temperature.
In general shelf-life of fresh-cut kale was 28, 14 and 5 days at 0, 5 and 10°C, respectively. Intact leaves generally had 7, 50 and 20% greater shelf life than fresh-cut leaves, respectively.

Controlled atmospheres of air + 7.5% CO2 and air + 15% CO2 had a significant impact in the postharvest performance of fresh-cut kale leaves compared to air storage at 5°C for 24 days. Marketable quality (except for odd-odors) and total chlorophyll content showed delayed rates of deterioration under these atmospheres. Conversely, total ammonia content increased notably with air + 15% CO2 atmosphere, which suggested the occurrence of internal deteriorative changes not consistent with visual and external indicators of the senescence process. Total MDA content was not affected by the use of these controlled atmospheres.

The use of a MAP of about 15% O2 + 5% CO2 for fresh-cut processed kale was significantly affected by storage temperature. Temperature abuse (10 and 20°C) of the microperforated packaged fresh-cut kale lead to oxygen depletion and high carbon dioxide (18%) concentrations. Product held at 0°C, however, maintained good quality over 12 days with little change in atmosphere composition.

Objective color, expressed in terms of L* value and Hue angle, and chlorophyll estimators (SPAD meter) were influenced by temperature and fresh-cut processing but not by leaf maturity. L* and SPAD meter values increased and Hue angle decreased, which was associated with a loss of green and increased leaf yellowing as a response of chlorophyll breakdown promoted by fresh-cut processing and increased storage temperatures. The different measurements of color were reasonably well correlated with chlorophyll concentrations.
6. FIGURES AND TABLES
FIGURES

Figure 1. Appearance of intact kale leaves cv. ‘Winterbor’ during storage at 0, 10 and 20°C for 6 days (Pictures from Experiment 1).
**Figure 2.** Changes in objective color values (L* and Hue angle) of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days, measured with two instruments. Each point represents the average of 4 replications per treatment. Each replication consisted of 6 kale leaves. Vertical bars represent standard error and are contained in the symbols when not shown. Different letters represent significant differences between treatments (combination of temperature and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 1).
Figure 3. Correlation of \( L^* \) measured with colorimeter to \( L^* \) measured with portable spectrophotometer of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days (Data from Experiment 1).

![Graph showing correlation between L* values measured with colorimeter and spectrophotometer. R^2 = 0.7810](image1)

Figure 4. Correlation of Hue angle measured with colorimeter to Hue value measured with portable spectrophotometer of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days (Data from Experiment 1).

![Graph showing correlation between Hue angle values measured with colorimeter and spectrophotometer. R^2 = 0.8394](image2)
Figure 5. Changes in total chlorophyll content (expressed in SPAD units) of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days. Each point represents the average of 4 replications per treatment. Each replication consisted of 6 kale leaves. Vertical bars represent standard error and are contained in the symbols when not shown. Different letters represent significant differences between treatments (combination of temperature and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 1).
Figure 6. Changes in total chlorophyll and total carotenoids content of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days. Each column represents the average of 4 replications per treatment, except for 0°C and 10°C stored for 6 days, with 1 and 2 replications, respectively. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (combination of temperature and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 1).
Figure 7. Correlation of total extracted chlorophyll (mg/g FW) to total chlorophyll content (SPAD units) measured with Chlorophyll meter SPAD of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days (Data from Experiment 1).
Figure 8. Appearance of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0, 5 and 10°C after 29, 18 and 11 days, respectively. FC: fresh-cut (Pictures from Experiment 2).
Figure 9. Changes in respiration of intact and fresh-cut kale leaves cv. ‘Winterbor’ stored at 0, 5 and 10°C for 29, 18 and 11 days, respectively. Each point represents the average of 3 replications per treatment for intact and fresh-cut leaves, respectively, except for intact immature leaves stored at 0°C, with 2 replications. Each replication consisted of one container of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 2).
Figure 10. Appearance of intact kale leaves cv. ‘Winterbor’ harvested at two maturity stages (Picture from Experiment 3).
**Figure 11.** Appearance of fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages during storage in unsealed bags in air at 7.5°C for 12 days (Pictures from Experiment 3).

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Figure 12. Changes in marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages and stored in unsealed bags at 7.5°C for 12 days. Scores were assigned based on a hedonic scale. Each point represents the average of 4 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown. Different letters represent significant differences between treatments (comparison between maturity stages at each evaluation time) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 3).
Figure 13. Changes in objective color (L* and Hue angle measured with colorimeter) of intact leaves and fresh-cut kale cv. ‘Winterbor’ harvested at two maturity stages and stored in unsealed bags at 7.5°C for 12 days. Each point represents the average of 4 replications per treatment. Each replication consisted of 4 kale leaves. Vertical bars represent standard error and are contained in the symbols when not shown. Different letters represent significant differences between treatments (comparison between maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 3).
Figure 14. Changes in total chlorophyll and total carotenoids content of fresh-cut kale cv. ‘Winterbor’ harvested at two maturity stages and stored at 7.5°C for 12 days. Each column represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (combination of maturity stage and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 3).
Figure 15. Changes in antioxidant capacity (determined by FRAP method) of fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages and stored at 7.5°C for 12 days. Each column represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (combination of maturity stage and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 3).
Figure 16. Changes in total ammonia content of fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages and stored at 7.5°C for 12 days. Each point represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown (Data from Experiment 3).
Figure 17. Appearance of intact kale leaves cv. 'Winterbor' harvested at three maturity stages during storage in air at 0 and 5°C for 42 and 28 days, respectively (Pictures from Experiment 4).
Figure 18. Appearance of fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage in air at 0°C for 42 days (Pictures from Experiment 4).
Figure 19. Appearance of fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage at 5°C for 28 days (Pictures from Experiment 4).
Figure 20. Changes in marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage at 0°C for 42 days. Scores were assigned based on hedonic scales. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 4).
Figure 21. Changes in marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage at 0°C for 42 days. Scores were assigned based on hedonic scales. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 4).
Figure 22. Changes in marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage at 5°C for 28 days. Scores were assigned based on hedonic scales. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 4).
Figure 23. Changes in marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages during storage at 5°C for 28 days. Scores were assigned based on hedonic scales. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 4).
Figure 24. Changes in total chlorophyll content of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each column represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 4).
Figure 25. Changes in total carotenoid content of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each column represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 4).
Figure 26. Changes in total ammonia content of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each point represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown (Data from Experiment 4).
Figure 27. Changes in total malondialdehyde (MDA) content of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each column represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 4).
Figure 28. Appearance of intact kale leaves cv. ‘Lacinato’ harvested at three maturity stages (Picture from Experiment 5).
Figure 29. Appearance of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively (Pictures from Experiment 5).
Figure 30. Changes in marketability attributes of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0°C for 42 days. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 5).
Figure 31. Changes in marketability attributes of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 5°C for 28 days. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 5).
Figure 32. Changes in objective color values ($L^*$ and Hue angle) of fresh-cut kale leaves cv. 'Lacinato' harvested at three maturity stages and stored at 0°C for 42 days. Each point represents the average of 3 replications per treatment. Each replication consisted of 4 kale leaves. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 5).
Figure 33. Changes in objective color values (L* and Hue angle) of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 5°C for 28 days. Each point represents the average of 3 replications per treatment. Each replication consisted of 4 kale leaves. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 5).
Figure 34. Correlation of $L^*$ measured with colorimeter to $L^*$ measured with portable spectrophotometer of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively (Data from Experiment 5).

Figure 35. Correlation of Hue angle measured with colorimeter to Hue angle measured with portable spectrophotometer of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively (Data from Experiment 5).
Figure 36. Changes in total chlorophyll and total carotenoids content of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each column represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 5).
**Figure 37.** Changes in total ammonia content of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each point represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown (Data from Experiment 5).

![Ammonia Content Graph](image)

**Figure 38.** Changes in total MDA content of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Each column represents the average of 3 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 5).

![MDA Content Graph](image)
Figure 39. Changes in respiration of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 38 and 24 days, respectively. Each point represents the average of 3 replications per treatment, except for immature leaves stored at 0°C, with 2 replications. Each replication consisted of one container of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 5).
Figure 40. Appearance of fresh-cut kale leaves cv. ‘Winterbor’ stored in three controlled atmospheres at 5°C for 24 days (Pictures from Experiment 6).
Figure 41. Changes in marketability attributes of fresh-cut kale leaves cv. ‘Winterbor’ stored in three controlled atmospheres at 5°C for 24 days. Scores were assigned based on hedonic scales. Each point represents the average of 4 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 6).
Figure 42. Changes in total chlorophyll and total carotenoids content of fresh-cut kale leaves cv. 'Winterbor' at three atmospheres stored at 5°C for 24 days. Each column represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among atmospheres at each evaluation time) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 6).
Figure 43. Changes in total ammonia content of fresh-cut kale leaves cv. ‘Winterbor’ at three atmospheres stored at 5°C for 24 days. Each point represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown (Data from Experiment 6).
Figure 44. Changes in total MDA content of fresh-cut kale leaves cv. ‘Winterbor’ at three atmospheres stored at 5°C for 24 days. Each column represents the average of 4 replications per treatment. Each replication consisted of 4g of kale. Vertical bars represent standard error and are contained in the columns when not shown. Different letters represent significant differences between treatments (comparison among atmospheres at each evaluation time) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 6).
Figure 45. Appearance of fresh-cut kale leaves cv. 'Winterbor' packaged in microperforated bags and stored at 0, 5 and 10°C for 20, 20 and 16 days, respectively (Pictures from Experiment 7).
Figure 46. Changes in marketability attributes of fresh-cut kale leaves cv. ‘Winterbor’ packaged in microperforated bags and stored at 0, 5 and 10°C for 20, 20 and 16 days, respectively. Scores were assigned based on hedonic scales. Each point represents the average of 3 replications per treatment. Each replication consisted of one bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 7).
Figure 47. Changes in CO2 and O2 concentrations of fresh-cut kale leaves cv. ‘Winterbor’ packaged in microperforated bags and stored at 0, 5 and 10°C for 20 days. Each point represents the average of 6 replications per treatment. Each replication consisted of one commercial bag of kale. Vertical bars represent standard error and are contained in the symbols when not shown (Data from Experiment 7).
Figure 48. General correlation of total chlorophyll content (mg/g FW) to total carotenoid content (mg/g FW) (Data from Experiments 1, 3, 4, 5 and 6).
### Table 1.

Means of color components (measured with two instruments) of intact kale leaves cv. ‘Winterbor’ stored at 0, 10 and 20°C for 6 days. Values represent the average of 4 replications per treatment. Each replication consisted of 6 kale leaves. Different letters represent significant differences between treatments (combination of temperature and day) by Tukey’s test, considering $\alpha=0.05$ (Data from Experiment 1).

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Table 2. Means of color components of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages and stored at 7.5°C for 12 days. Measurements were made with a colorimeter. Values represent the average of 4 replications per treatment. Each replication consisted of 4 kale leaves. Different letters represent significant differences between treatments (comparison between maturity stages at each format at each evaluation time) by Tukey’s test, considering $\alpha=0.05$. FC: fresh-cut (Data from Experiment 3).

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**Table 3.** Means of chlorophyll, carotenoids, ammonia and antioxidants content of fresh-cut kale leaves cv. ‘Winterbor’ harvested at two maturity stages and stored at 7.5°C for 12 days. Values represent the average of 4 replications per treatment. Each replication consisted of 4g of kale. Different letters represent significant differences between treatments (comparison between maturity stages at each evaluation time) by Tukey’s test, considering α=0.05 (Data from Experiment 3).

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<th>Chl b (mg/g FW)</th>
<th>Total chlorophyll (mg/g FW)</th>
<th>Total carotenoids (mg/g FW)</th>
<th>Ammonia (mg/g FW)</th>
<th>Total antioxidant capacity (mmole Fe²⁺/100g FW)</th>
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Table 4. Means of marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0°C for 42 days. Scores were assigned based on hedonic scales. Values represent the average of 3 replications per treatment. Each replication consisted of one bag of kale. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering α=0.05. FC: fresh-cut (Data from Experiment 4).

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<th>Yellowing 1= None 5=Severe</th>
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Table 5. Means of marketability attributes of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 5°C for 28 days. Scores were assigned based on hedonic scales. Values represent the average of 3 replications per treatment. Each replication consisted of one bag of kale. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering α=0.05. FC: fresh-cut (Data from Experiment 4).

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Table 6. Means of total ammonia content of intact and fresh-cut kale leaves cv. ‘Winterbor’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Values represent the average of 3 replications per treatment. Each replication consisted of 4g of kale. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering α=0.05. FC: fresh-cut (Data from Experiment 4).

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Table 7. Means of marketability attributes of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Scores were assigned based on hedonic scales. Values represent the average of 3 replications per treatment. Each replication consisted of one bag of kale. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each temperature) by Tukey’s test, considering α=0.05 (Data from Experiment 5).

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Table 8. Means of color components of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Measurements were made with a colorimeter. Values represent the average of 3 replications per treatment. Each replication consisted of 4 kale leaves. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering α=0.05 (Data from Experiment 5).

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Table 9. Means of color components of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Measurements made with a portable spectrophotometer. Values represent the average of 3 replications per treatment. Each replication consisted of 4 kale leaves. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering \( \alpha = 0.05 \) (Data from Experiment 5).

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<th>b*</th>
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Table 10. Means of total ammonia content of fresh-cut kale leaves cv. ‘Lacinato’ harvested at three maturity stages and stored at 0 and 5°C for 42 and 28 days, respectively. Values represent the average of 3 replications per treatment. Each replication consisted of 4g of kale. Different letters represent significant differences between treatments (comparison among maturity stages at each evaluation time at each format) by Tukey’s test, considering α=0.05 (Data from Experiment 5).

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Table 11. Means of marketability attributes of fresh-cut kale leaves cv. ‘Winterbor’ at three atmospheres stored at 5°C for 24 days. Scores were assigned based on hedonic scales. Values represent the average of 4 replications per treatment. Each replication consisted of one bag of kale. Different letters represent significant differences between treatments (comparison among evaluation times at each atmosphere) by Tukey’s test, considering α=0.05. Atm: atmosphere (Data from Experiment 6).

<table>
<thead>
<tr>
<th>Atm</th>
<th>Storage time (Days)</th>
<th>Off-odors 1=None 5=Severe</th>
<th>OVQ 1=Unusable 9=Excellent</th>
<th>Yellowing 1=None 5=Severe</th>
<th>Decay/Deterioration 1=None 5=Severe</th>
<th>Discoloration/Browning 1=None 5=Severe</th>
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</thead>
<tbody>
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<td>1.00c</td>
<td>1.00c</td>
</tr>
<tr>
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<td>8.00b</td>
<td>2.00bc</td>
<td>1.00c</td>
<td>1.00c</td>
</tr>
<tr>
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<td>7.00c</td>
<td>2.00bc</td>
<td>1.00c</td>
<td>2.00b</td>
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<tr>
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<td>2.00b</td>
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</tr>
<tr>
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<td>4.25a</td>
<td>4.00a</td>
<td>4.00a</td>
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<td>9.00a</td>
<td>1.00c</td>
<td>1.00b</td>
<td>1.00c</td>
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<td>2.00b</td>
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<td>4.00e</td>
<td>3.50a</td>
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</tr>
<tr>
<td>15% CO2</td>
<td>0</td>
<td>1.00d</td>
<td>9.00a</td>
<td>1.00b</td>
<td>1.00b</td>
<td>1.00c</td>
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<td>1.00b</td>
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<td>5.00d</td>
<td>3.00a</td>
<td>2.00a</td>
<td>3.00a</td>
</tr>
</tbody>
</table>
Table 12. Means of total chlorophyll, carotenoids, ammonia and MDA content of fresh-cut kale leaves cv. ‘Winterbor’ at three atmospheres stored at 5°C for 24 days. Values represent the average of 4 replications per treatment. Each replication consisted of 4g of kale. Different letters represent significant differences between treatments (comparison among evaluation times at each atmosphere) by Tukey’s test, considering $\alpha=0.05$. LSD test was performed for total chlorophyll, Chl a and Chl b, considering $\alpha=0.05$. Atm: atmosphere (Data from Experiment 6).

<table>
<thead>
<tr>
<th>Atm</th>
<th>Storage time (Days)</th>
<th>Chl a (mg/g FW)</th>
<th>Chl b (mg/g FW)</th>
<th>Total chlorophyll (mg/g FW)</th>
<th>Total carotenoids (mg/g FW)</th>
<th>Ammonia (mg NH4/g FW)</th>
<th>MDA (nmol/g FW)</th>
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</thead>
<tbody>
<tr>
<td>Air</td>
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<td>0.77a</td>
<td>0.28a</td>
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<td>0.17a</td>
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<td>0.15a</td>
<td>0.0027b</td>
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<tr>
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<td>34.06a</td>
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<tr>
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<td>0.24ba</td>
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<td>0.0061a</td>
<td>36.20a</td>
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<td>0.75c</td>
<td>0.14a</td>
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<tr>
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<td>0.33a</td>
<td>1.11a</td>
<td>0.16a</td>
<td>0.0024b</td>
<td>34.81a</td>
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<td>0.0092ba</td>
<td>32.40ba</td>
</tr>
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<td>0.0314ba</td>
<td>30.78b</td>
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<td>0.16a</td>
<td>0.0350ba</td>
<td>31.32ba</td>
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<td>0.31a</td>
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<td>0.18a</td>
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</table>
Table 13. Means of marketability attributes of fresh-cut kale leaves cv. ‘Winterbor’ stored at 0, 5 and 10°C for 20, 20 and 16 days, respectively. Scores were assigned based on hedonic scales. Values represent the average of 3 replications per treatment. Each replication consisted of one bag of kale. Different letters represent significant differences between treatments (comparison among evaluation times at each temperature) by Tukey’s test, considering α=0.05 (Data from Experiment 7).

<table>
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<tr>
<th>T(°C)</th>
<th>Storage time (Days)</th>
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<th>OVQ 9= Excellent 1= Unusable</th>
<th>Yellowing 1= None 5= Severe</th>
<th>Decay/ Deterioration 1= None 5= Severe</th>
<th>Discoloration/ Browning 1= None 5= Severe</th>
</tr>
</thead>
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<td>2.00a</td>
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7. REFERENCES


