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

EFFECT OF MINIMAL PROCESSING STEPS AND OPERATION MODES ON QUALITY OF LEAFY VEGETABLES

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DEDICATION

To the Glory of the Almighty God.

Abstract

Minimal processing and storage of leafy vegetables are limited by several quality challenges despite numerous research interventions. Some studies have assessed pre-harvest and postharvest effects on quality of raw material and sometimes also on quality and shelf-life of the resulting processed product, however, the information on effect of the processing steps on its quality is lacking. This study, assessed the effect of operation modes, processing steps and storage on the nutritional, sensorial and microbial quality of minimally processed rocket leaves and cut lettuce. Rocket leaves and Lettuce (Iceberg and Romaine lettuce) samples were collected during processing from 4 and 2 processors respectively, by sampling the product flowing after each main processing steps (from raw material to washing drying and packaging). In addition processors belonged to Italy (Puglia) and USA (California and Arizona), and particularly 2 processors for rocket leaves and one for cut lettuce (Iceberg lettuce for Italy and Romaine lettuce for USA). Processors differed in their mode of operation and mainly by washing plant and design, type of sanitizer used and drying equipment. The effect of operation modes were evaluated on different quality attributes. For experiments in Italy, Total soluble solids (TSS), Titratable acidity (%TA), pH, Total phenols (TP), Total antioxidant activity (TAA), Ascorbic acid (AA), Dehydroascorbic acid (DHAA), Total vitamin C (Vit. C), Mesophilic count (MC), Psychrophilic count (PC), Yeast and molds (Y&M) and aroma volatiles of products after each processing step, including storage at 5 ^{o}C, were evaluated. For the experiments conducted in the USA, the effect of operation modes was evaluated on TP, TAA, MC, PC, Y&M, glucose, sinapic acid and ferulic acid (rocket leaves) as well as chlorogenic acid and p-Coumaric acid (romaine lettuce), with RT-PCR confirmatory tests for screening total bacteria.

Results obtained from common steps and quality attribute were evaluated with a 2-Way ANOVA design and random effect, and by applying a multivariate clustering technique in order to detect variation due to different processors and processing steps. Moreover results of each processor were individually analyzed for each processing step. Results showed that raw material quality influenced final product quality. For rocket leaves irrespective of location, the washing and sanitization steps increased phytonutrients properties of leaves (TP, TAA, AA, Total Vit. C sinapic and ferulic acids), inducing a reduction of microbial counts (MC, PC, Y&M), except for one case (processor C) where cross-contamination occurred. The washing steps that significantly increased TP also influenced the release of stress related volatiles (2-Methyl furan, Benzaldehyde, Dimethyl sulphide and (Z)-2-Penten-1-ol), for- Processor A whereas for Processor B, the stress related volatiles decreased (mostly aldehyde and sulphur compounds) when the increase in TP was not substantial during washing. Generally this increase was not affecting the overall nutrient content of the final product since a further oxidation was then induced by the drying steps (spin or tunnel drying). Passive MAP storage caused increase ($p \leq 0.05$) in DHAA, Y&M with high level ketone, sulphur compounds and aldehyde indicating early senescence where more stress was induced during processing, but did not significantly affect other phytonutrient, an increase of antioxidants was

observed, most probably as a defense response to the growth of PC and Y&M which also induced an increase of the production of benzaldehyde, methyl thiocyanate and dimethyl sulphide.

For lettuces, operation modes and processing steps of processors of iceberg and romaine caused a decrease in both phytonutrients and microbial counts (MC, PC, Y&M), but with some differences. The cutting of iceberg lettuce induced the release of alcohols, aldehydes and dimethyl sulphide while the cut surface of romaine had increased levels of all microbial counts probably from a contamination of the cutting equipment. In both cases nonetheless, the washing steps reduced the emission of volatiles and microbial counts respectively. Active MAP storage for 3 days in the case of iceberg lettuce led to a decrease in AA, DHAA, but a rise in TP, TAA, MC, PC, Y&M and volatiles already identified after drying. Moreover, microbial counts remained lower than that of the raw material prior to processing. Glucose content increased in Romaine lettuce after cutting but decreased during futher processing. TSS, %TA and pH were not considerably affected by the processing steps.

A further study on the effect of the drying conditions on quality of rocket leaves after storage was also carried out. Temperature, belt speed and time were regulated to achieve two air-drying treatments leading to a residual surface water reduction below 2%, namely T-A (33^{o}C, 0.022m/s and 5.37mins) and T-B (40^{o}C, 0.026m/s and 4.45mins). Air ventilation was maintained at 100% in both treatment combinations. Raw material prior to processing was sampled and tested for sensory and microbial quality, while rocket leaves soon after the drying (achieving residual surface moisture of 1.9%-T-A and 0.4%-T-B), were directly packaged in polypropylene film bag (passive modified atmosphere) and stored for 5, 9 and 15 days at 5^{o}C. The stored samples were analyzed in triplicates for colour changes, TSS, pH, %TA, Total chlorophyll (TC), Total Vit. C (AA and DHAA), TP, TAA, sensory quality, MC, PC and Y&M and aroma volatiles. Two-way anova results showed that although L*, b* and chroma angle showed differences ($p \leq 0.05$) in T-A and T-B , the overall change in colour ΔE was due to the storage period (15days), but was not visually perceptible. %TA increased with decrease pH after 5days, while TSS increased for both treatments during storage. Raw material sensory quality decreased after minimal processing which also was effective in reducing microbial load; no major significant differences between T-A and T-B drying treatment during the storage period were observed. Passive MAP packaging and cold storage temperature minimized quality changes over time. Slight higher water surface of rocket leaves from the treatment at the lowest temperature (T-A), may have affected microbial and tissue disintegration in storage inducing the production of 2-Ethyl furan, but without inducing sensible variation in sensorial quality. Phytonutrients were also maintained during the storage period, with the exception of TC which reduced by 9.43% and 13.27% for T-A and T-B and TP which reduced by 4.32% for T-A. MC and PC reached the limit for spoilage after 15 days of storage while yeasts and molds growth exceeded the limit for spoilage after 9 days.

These results suggested that the treatment at 40 °C and shortest time should be preferred in order to increase process productivity, allowing to maintain lower residual water without inducing any thermal degradation.

It can be concluded that minimal processing steps, the mode of operation of different processors, the type of product and storage conditions influenced the final product quality at varying degrees. Generally the washing reduced microbial counts, inducing a stress that in whole rocket leaves favoured an increase phytonutrient, while in cut lettuce as also in consequence of cutting a more pronounced oxidation was observed. **All these finding contributed to fill the existing gap between physiology of cut tissues and general knowledge about the impact of processing operations on quality of the final product, by increasing the amount of detailed information which can be available for processors.**

Effect of minimal processing steps and operation modes on quality of leafy vegetables

TABLE OF CONTENTS

PART ONE: GENERAL

1.0 Review paper 1: Effects of Harvesting and Postharvest handling of raw material on the quality of fresh cut products

1.1 Introduction

1.1.1 Background and Importance of fresh cut products

Fresh fruits and vegetables are known to be beneficial for human health and wellbeing (Liu, 2003; European Commission 2006; Cooper *et al.*, 2012). This health- promoting quality of vegetables are associated with the composition of phytochemicals (including, vitamins C, A and E) or bioactive substances necessary for stimulation of desired metabolic processes (Nurzynska-Wierdak, 2015). Phytochemicals include the phenolic and carotenoid compounds which have been well studied (Liu, 2004), in addition to nitrogen-containing (e.g. polyamines) and organosulfur compounds (e.g. isothiocyanates) which are currently receiving attention.

Globally, an estimated 1.7million annual deaths have been associated with low intake of fruits and vegetables (Lim *et al.*, 2012); current studies have shown that low affordability may have largely contributed to this situation (Miller *et al.*, 2016). Even so, this cost constraint is sometimes related to the high perishability of fruits and vegetables limiting their efficient use and accessibility. Overall, the situation varies depending on country specific circumstances, environmental and handling conditions as well as socioeconomic status of individuals. Typically, among some Europeans, perishable nature of healthy foods are not considered as critical barriers to healthy eating, however, busy lifestyle and irregular working schedules have contributed to low consumption of vegetables (Kearney and McElhone, 1999; Pollard *et al.*, 2002).

In recent years, minimal processing and ready-to- eat food industries have provided a means for enhancing the consumption of fruits and vegetables, maintained its freshness for considerable periods and eased distribution through convenient preparation and packaging. For instance, bulky produce like spinach, lettuce and other broad leaves are minimal processed or shredded (size reduction) and packaged

to ease distribution; similarly fruits like litchi and pomegranates are peeled and prepared to enhance their consumption. Resultantly, this product segment's share of global fruit and vegetable industry revenue has grown over the past five years (ibis world report, 2017). Product range in this sector include fruits cups, ready to eat salads and pre-cut vegetables (ibis world report, 2017) whichare mainly sold in supermarkets, fast food joints, restaurants, food vending areas of airports or are served to passengers during flights.

In Italy for example, an 8.3% increase in the consumption of ready to eat vegetables was observed between January and October 2010, whereas consumption of fruits and vegetables decreased by 0.8% in the same period (ISMEA, 2010; D'Acunzo *et al*., 2012). Also from July 2011 to July 2013, the number of households which consumed fresh-cut products increased from 15.8 million to 17 million (Nielsen, 2013). Domestic sales continued to rise reaching a rate of 6.4% between August 2014 and August 2016 amounting to 90,000 tons (ISMEA-Nielson, Consumer surveys).

Though the market for minimal processed (fresh-cut) product is growing, the raw material are sourced from different production and produce aggregation hubs and require distribution through various channels. As a result, different variables associated with produce harvesting, like maturity stage, handling, transportation and storage procedures may have enormous impact on the quality of intact raw material and hence on the minimal processed products.

1.1.2 Challenges in the fresh-cut product industry

Fruits and vegetables begin to senesce through metabolic, biochemical and enzymatic reactions, immediately after harvest, resulting from harvest induced stress, temperature, respiration and ethylene production which causes changes in cell walls, plant hormones and phytochemicals (Paliyath and Murr, 2008; Siomos and Koukounaras, 2007). This situation may be worsened during the postharvest chain if the management of the storage factors is not completely under control.

Moreover, the coring, peeling, dicing and slicing activities aggravate this situation and may affect texture, flavor and color/ appearance of the final products. Typically, discoloration of minimally processed garlic is found in correspondence of damaged areas (Cantwell *et al*., 2001). Also whitening in carrots (Simões *et al*., 2010) and beet roots (Vitti *et al*., 2005) occurs due to dehydration from peeling and cutting. In spinach and apple water losses of 3 and 5% respectively renders the products unmarketable (Sams, 1999). Other fresh-cut processing challenges include discoloration of cut-surfaces of sun choke artichokes (Wang and Cantwell, 2015) and Catanese artichokes (Amodio *et al*., 2011); translucency in fresh-cut pineapples (Hotegni *et al*., 2014); leakage in tomato slices, leakage and softening in water melon cubes (Gorny, 1997); browning, softening, surface dehydration, off-flavor and off-odor development in fresh-cut Fuji apples (Salvia-Trujillo *et al*., 2015); and the destruction of the cell-wall leading to soften and loss of water in white cabbages (Toivonen and Brummell, 2008).

1.1.3 Quality of the raw material is the key

Raw materials used in the minimal processed industry has a major influence on the final product quality and therefore it should fulfil consumer expectations of freshness, flavour, convenience, microbial and nutritional quality (Floristán, 2009). However, several factors influence the raw material quality, which include the variety, production practices, maturity at harvest, temperature, relative humidity and physical damage during handling and transportation as well as storage conditions prior to processing. These factors may influence the metabolic activity, respiration rate, biochemical changes and microbial growth which may in turn affect appearance, color, texture and flavor quality of raw material. Typically, products with higher respiration rates would rapidly lose acids, sugars and other constituents that determine taste, flavor and nutritional quality losses (Cantwell and Suslow, 2002). In addition some inherent raw material characteristics may contribute to its quality. For example, levels of sugars in terminal and lateral heads of broccoli was found to vary depending on cultivar (Rosa *et al*., 2001). Some produce have improved resistance to stress like cutting and the resulting browning during minimal

processing activities. For instance, Sabina and Eden apple cultivars have low or non-browning cultivar properties (Hampson *et al*., 2006; Khanizadeh *et al*., 2006), probably due to their resistance to oxidative stress and increased respiratory effects. To ensure optimal quality of minimally processed products, it is important to monitor cultivar characteristics of the raw material, besides ensuring that harvest and postharvest handling methods does not impact damage that will affect the final product quality.

1.1.4 General processes and mechanism leading to changes in quality of raw material

Raw material meant for the fresh-cut industry are produced in open fields, greenhouses or high tunnel structures. They are harvested, packaged and transported directly to short-distance processing companies or for long distance, within or outside the country, either for processing or for market distribution. The latter case include exotic and out-of-season produce that have to be shipped for fresh consumption and minimal processing in other countries. Figure 1, describes the differences in handling processes for both cases before they are used. Produce meant for short distance processing companies (mostly processed within 3 days) (Figure 1.1A) are usually harvested at optimum maturity for the desired organoleptic properties while products that are shipped for long distances (Figure 1.1B), are harvested at commercial maturity allowing that desired characteristics can be maintained up to the final destination and handling . However, handling processes are of prime importance for obtaining high quality cut products. This is because there are potential variables from harvesting, handling, transportation and storage procedures, which may have enormous impact on the quality of intact raw material and hence on minimal processed products.

Naturally, fruits and vegetables begin to senesce through metabolic, biochemical and enzymatic reactions, immediately after harvest, resulting from changes in cell walls, plant hormones and phytochemicals; as mentioned, natural senescence processes may be accelerated in case of non adequate handling during the

postharvest chain (Wills *et al*, 2007; Batu, 2008; Workneh and Osthoff, 2010). Changes occur in relation to respiration, ethylene production, phenolic metabolism and lignin formation which may lead to compositional and physical changes. While fresh produce with physical changes in color and appearance can be eliminated during sorting, compositional changes may not be easily detected and can affect quality of the minimal processed product. For example, increased respiration and temperature stress may lead to reduced carbohydrates (starch to sugar conversion; desirable in apples and banana but not potatoes), organic acids, ascorbic acids and low flavor quality (Kader, 2002); ethylene production especially in climacteric produce may initiate chlorophyll degradation (desirable in fruits and not in vegetables), anthocyanin synthesis, ripening and softening, and phenolic metabolism may lead to either polyphenol oxidase activity and browning or phenylalanine activity leading to lignin formation and cell wall thickness (texture changes e.g toughening in asparagus spears and root vegetables) (Saltveit, 1997) reducing the wholesomeness of the raw material. In addition to the changes in colour, texture, flavor and appearance, physiological processes influences deterioration by enzymes (Rahman, 2007). Enzymatic activities causes biochemical changes which is aggravated by oxidative stress.

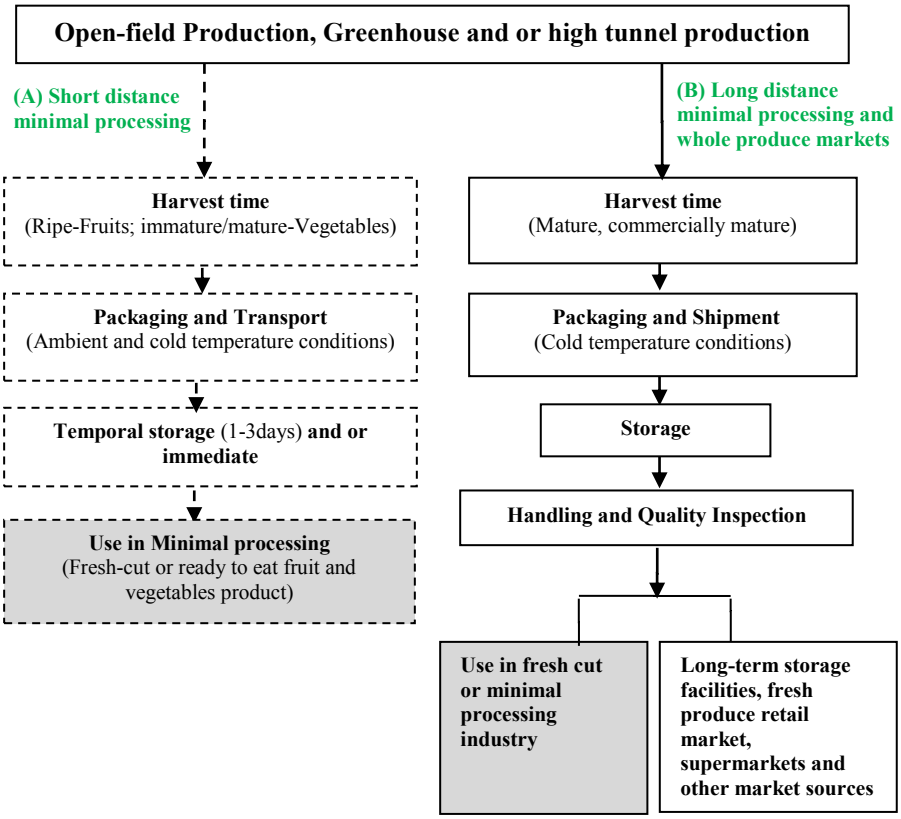


Figure 1.1: Harvest and Postharvest handling stages of raw material for minimal processing

Oxidative stress (which occurs mainly in chloroplast, mitochondria and peroxisomes) are also caused by mechanical impacts from handling and increased respiration which leads to biochemical changes and loss of phytochemicals. Under oxidative stress, reactive oxygen species (ROS) are metabolically generated in the cells causing an upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) and the activity of free radical scavengers which consist of non-enzymatic antioxidants like vitamins A (B-carotene), vitamin C (Ascorbate), Vitamin E (α-tocopherol), glutathione, flavonoids, phenols and minerals that are redox cycling (Dröge, 2002), Figure 1.2. A balance between the production and scavenging of ROS may occur, leading to redox homeostasis (Poljsak *et al*., 2013). However, continue stress may result in breakage of homeostasis balance. For instance, tannin production due to antioxidant

activity may cause internal browning of raw materials like pears and artichoke which have to be discarded during minimal processing. Internal bruises may also cause disruption in the volatile biosynthetic pathway leading to pre-mature synthesis of the secondary aroma compounds and loss of flavor as have been reported for tomatoes (Moretti *et al*., 2002). This process is even more aggravated by coring, peeling, dicing and slicing activities during minimally processing which may affect texture, flavor and color/ appearance of the final products (Barrett *et al*., 2010).

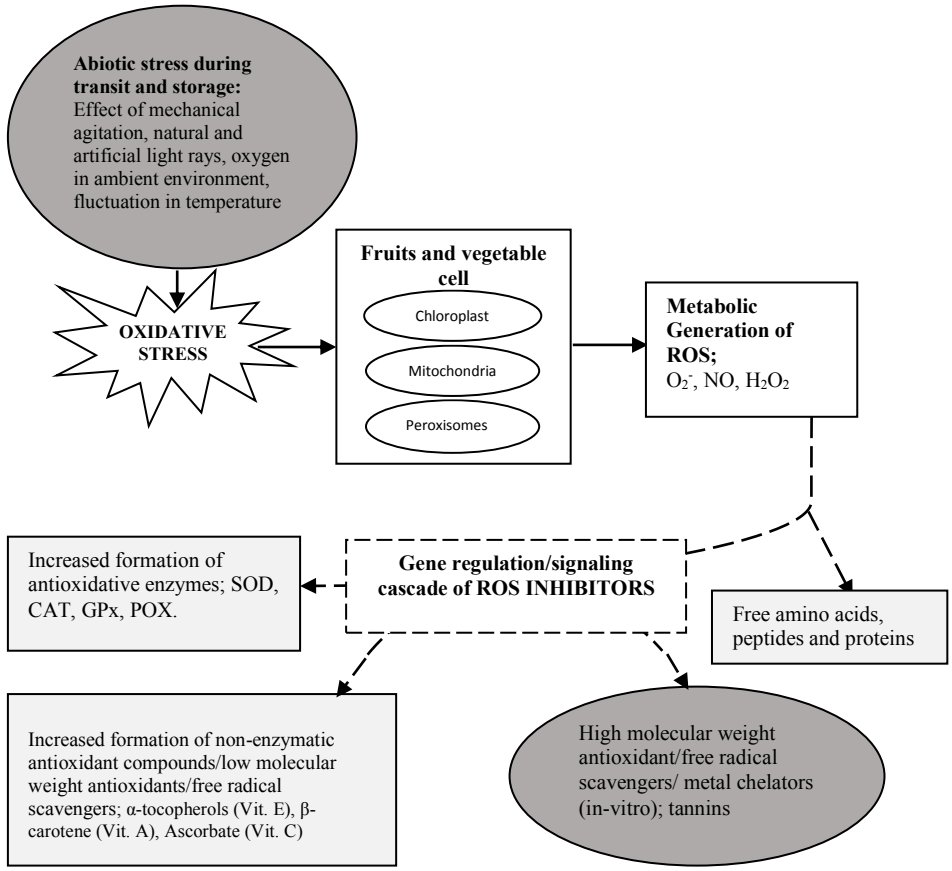


Figure 1.2: Mechanism for breakage of redox homeostasis balance of fruit and vegetable due to postharvest handling stress.

This paper seeks to review existing literature on biochemical, physiological and enzymatic changes as affected by harvest, maturity and handling of fruits and vegetables meant for the minimal processing industry in order to point out areas for further research with the aim of enhancing the sensorial, nutritional and biochemical quality of such products.

1.2. Harvesting related factors affecting raw material quality

Harvesting procedures required for optimum quality of fruits and vegetables meant for fresh consumption may not be suitable for fresh-cut or minimal processing. Minimal processing inflicts more stress on the product tissues, resulting in higher perishability of cut product compared to the whole one. Furthermore, harvesting maturity stage (colour, size, shape, firmness, head length, width, compactness for vegetable heads and/or number of leaves), harvesting procedure (manual or mechanical, cutting, hand pulling or twisting) and time of harvesting (during the season or the day) may affect minimal processing quality. This section will discuss the impact of harvesting on fresh produce and their corresponding minimal processed product quality.

1.2.1 Effect of harvest maturity

The maturity stage of fruits and vegetables at harvest influences the level of stress tolerance and the quality of minimally processed products, especially if cut. This is because the ability of the plant to withstand any postharvest handling is based on its characteristics at harvest. Harvest maturity stage suitable for fresh cut processing depends among others factors on whether the produce is climacteric or non-climacteric. While most vegetables are non-climacteric, due to their low levels of ethylene production, the situation varies in fruits due to physiological differences. Tudela *et al.* (2013), studied the effect of harvest maturity stage on the quality of fresh-cut iceberg lettuce and found better appearance and less cut-edge browning of heads processed at the immaturity stages versus the matured ones, though the immature ones had high correlation with the production of off-odors. Significantly higher soluble solids, total phenols and lowest nitrates have also been reported for

younger rocket leaves with no differences in shelf-life when compared to older leaves (Watada *et al.* 1996). Baby leaf spinach harvested one week before commercial maturity showed higher vitamin C content and better quality than leaves harvested at commercial maturity (Bergquist *et al*., 2006). Similarly, higher ascorbic acid concentration were found in small cabbage heads than large ones (Weston and Barth, 1997). Furthermore, more thiamine and riboflavin were found in small turnip (Brassica rapa L.) leaves but less carotene than large leaves (Salunkhe *et al*., 1991). In the case of multi-leaf lettuce the situation is different, more mature leaves were found to produce firmer cut products, with longer shelf-life (Martínez-Sánchez *et al*., 2012). However, in relation to lettuce heads, it should be noted that the rate of browning is higher in over mature heads compared to mature and immature heads (Gil *et al*., 2012).

In fruit vegetables, a different scenario is observed. Marin *et al.* (2004) comparing phytochemical content of peppers at different maturity stages, found out that the optimum maturity stage for the highest vitamin C and carotenoid content was the red ripe stage with 93mg$100g^{-1}$ and 838.80 mg$100g^{-1}$ respectively, while fruits harvested at an immature stage, had profound higher content of polyphenols (hydroxycinnamates and flavonoids). High vitamin C is a key quality measure while polyphenol production is an indication of tissue stress and possible senescence, thus making the red ripe stage a better option for fresh-cut processing. In addition it has been reported that slices from fully colored red bell peppers have retained better post-cutting quality, lower respiration rate and higher soluble solid content than slices from mature-green bell peppers (Piazzolla *et al.*, 2012). This was also found for tomatoes; slices at the pink and red ripening stage had less firmness decay (texture loss) compared to that of the breaker stage during storage at 20°C (Lana *et al*., 2005). It could be deduced that leafy vegetables and fruit vegetables may have contrasting maturity requirement for minimal processing.

As for fruits, ripening contributes to achieving high organoleptic properties of the cut product but it may result in a reduction of the shelf-life. Overripe peach and nectarine slices had preferable organoleptic quality but considerably shorter shelf-life than slices from green fruits (Gorny *et al*., 1998). Interestingly, fruits are mostly climacteric and hence desirable quality attributes of flavor, taste and color can be attained even when minimally processed at half-ripe stage due to their ability to continue to ripening after harvest. Studies on fresh-cut slices made from half-ripe (13-16% soluble solids) mangoes, showed that, they maintained an acceptable appearance, texture, and taste, which continued to develop during post-cutting life at 5^{o}C for 8days (Allong *et al.*, 2000).

Likewise, remarkable flavor differences were reported for mature-green versus ripe mangoes (Bender *et al.*, 2000). Fresh-cut slices obtained from firm-ripe mangoes were limited to 11 days due to development of off odor and desiccation while those from soft-ripe mangoes had a shelf-life of 7days, limited by poor texture, loss of characteristic flavor and discoloration (Beaulieu and Lea, 2003). . However, this may be cultivar dependent. Among Keitt, Tommy, Van Dyke, Haden and Palmer mango cultivars alone, unique differences exist in their starch degradation to sugars (Bernardes-Silva *et al.,* 2003). In Keitt mangoes, for instance, high starch content has been found at harvest maturity but degrades immediately after harvest probably due to the high α-amylase and β-amylase activity (Silva *et al.*, 2008). As a result, cubes from soft-ripe Kiett variety of mango which had initial lower soluble solid content compared to Palmer variety had higher soluble solid content after 4 days storage in MAP (Beaulieu and Lea, 2003). Similar to the Keitt mangoes, sweetness of fresh-cut cantaloupe cubes obtained from fruits harvested at $\geq$ ½ slip maturity, increased through storage (Beaulieu *et al.*, 2004). The author related this to the sweet/fruity flavor descriptors to the increase of non-acetate esters. On the other side it has been observed that fresh-cut honeydew melons with 13% soluble solids had better flavor and slower deterioration rate than that of cut melons with 8.8% soluble solids after 7days at 5 ^{o}C based on appearance, shear force values and taste scores (Watada and Qi, 1999).

Also, slices of conference pears minimally processed at partial ripe maturity stage and stored at 4°C presented more suitable characteristics for fresh-cut processing than slightly under-ripe pears which, on the other side exhibited less browning and firmness loss (Solvia - Fortuny *et al.*, 2004). It is evident that the maturity stage influences colour (browning potential), flavour, texture and taste of minimal processed product. Notably, partially ripe fruits may better resist firmness degradation and seem to be suitable for minimal processing compared to fully ripe, since the latter ones have a heighten level of beta-D-galactosidase (EC 3.2.1.23) leading to enhanced ripening-related cell wall disassembly; tissue exudation or browning in some cases (Solvia-Fortuny *et al*., 2004; Toivonen and Brummell, 2008). In addition, β-galactosidase (β gal, EC 3.2.1.23), hastens softening of fresh-cut tissues by altering the reactivity of cell wall polysaccharide to other cell wall hydrolases catalyzing the removal of terminal non reducing β-D-galactosyl residues from galactan, which then accelerate cell wall disassembly (Toivonen and Brummell, 2008; Li *et al.*, 2010).

Despite the basic importance of physiological maturity, in there is much uncertainty in regard to the stage of maturity at which quality cut products can be attained due to crop and cultivars differences. Therefore it is very necessary to identify the appropriate maturity stage and specific cultivar properties by which biochemical, enzymatic and phytochemical characteristics can provide product with optimal flavor, taste, color, firmness to be used in fresh-cut processing. Table 1 outlines the current harvest maturity indexes, the type of processing operations and the enzymes involved in quality losses after cutting of several species of vegetables (1.1 a) and fruits (1.1 b) with the attempt to give an overview of the main degradative reactions to be considered to determine the optimum maturity for minimally processing.
Furthermore, with increasing interest in underutilized and unconventional fresh-cut products it is vital that future research on harvest quality includes such products to promote diversity and sustain this processing sector.

Table 1.1: Harvest Indices, minimal processing activities and enzymatic reactions causing loss of organoleptic quality.

a. Vegetables

Raw Material	Existing harvest indices	Minimal Processing Activity	Enzymes	Reaction	Results
Broccoli	Compact bud cluster; florets closed	Cutting into florets	Lipoxygenase, Chlorophyllase, Chlorophyll oxidase Cysteine lyase (Derbali *et al*, 1998; Barrett *et al*., 2000)	Cleavage of phytol ring from chlorophyll; Degradation of chlorophyll; sulphur compound production	Loss of green color/yellowing and production of off-odors
Cabbage (Green and Red)	Firm and compact head with smooth leaves	Trimming of wrapper leaves, coring and cutting into shreds or diced	Lipoxygenase, Chlorophyll oxidase, chlorophyll peroxidase	Membrane fatty acid degradation due to lipoxygenase, leading to chlorophyll losses (Ch´eour *et al*., 1992). Interaction of peroxidase with phenolic compounds to produce superoxide anions which can directly oxidize chlorophylls (Yamauchi, *et al*. 2004)	Browning, off-flavors and off-odors
Carrot	Crispiness and size	Abrasion or peeling and cutting into baby carrots, shreds, sticks, slices, dices	Lipoxygenase Peroxidase, Syringaldazine oxidase (an isoform of peroxidase)	Oxidation of carotene;, oxidation of syringaldazine to lignin by Syringaldazineoxidase (Goldberg *et al*., 1985)	Change in colour; synthesis of lignin, 'white blush' on cut surfaces, ,.
Lettuce head	Size, firmness,compactness	Cutting into quarters, chopping into shreds or hand tearing	Phenylalaninelyase, Polyphenoloxidase	Synthesis of phenols due to Phenylalaninelyase activity; subsequent oxidation of phenols by polyphenoloxidase activity,	Cut edge browning
Lettuce, Romaine	Size, number of leaves	Chopping, hand tearing or hand separation whole leaf	Chlorophyllase Polyphenoloxidase	Degradation of chlorophyll; Oxidation of polyphenols	Reduction of green color and loss of phenolic content
Onion Bulb	Dryiness of leaves , beginning of top drying	Slivering, dicing, or cutting into slices, rings or chunks	Alliinase	Alliinase cleave isoalliin and other S-alk(en)ylcysteine sulfoxides to produce 1-propenyl- containing thiosulfanates, that in turn reacts with free amino acids (Kubec, *et al*. 2004); cellular disruption (Forney, 2015)	Changes in texture, aroma, and discoloration of wounded tissue
Spinach	Size, mid-maturity to young stage, fully turgid, uniformly green	Cutting or hand separation of whole leaves	Peroxidase Chlorophyll oxidase Cysteine lyase (Derbali *et al*, 1998; Barrett *et al*., 2000)	Peroxidase mediated breakdown of chlorophyll (Yamauchi and Watada,1991); volatile secondary metabolism	Loss of green color/yellowing and production of off-odors tissue softening

b. Fruits

Raw Material	Existing harvest indices	Minimal Processing Activity	Enzymes	Reaction	Results
Melons	Development of an abscission layer between the vine and the fruit peduncle	Peeling, slicing and cubing	Polygalacturonase Pectin esterase	Hydrolysis of ester bonds , between adjacent polygalacturonic acid residues in pectin	Juice leakage, tissue softening, translucency (glassiness)
Papaya	skin yellowing (55-80% yellow) Paul and Chen; Jayathunge, *et al*. 2014)	Peeling and cutting into slices, cubes or spheres	Polygalacturonase Proteases; pectin esterase-2006); Ascorbic acid oxidase	Hydrolysis of ester bonds between adjacent polygalacturonic acid residues or galacturonans in pectin; Hydrolysis of protein; Oxidation of ascorbic acid	Tissue softening; Loss of nutritional value and increase or decrease in digestibility.
Kiwifruit	6.5% to 14% soluble solid content; firmness	Peeling and Slicing	Polygalacturonase pectin methyl esterase	Solubilisation of pectin through hydrolysis of α-1, 4-glycosidic bonds indicative of pectin methyl esterase activity; which start few minutes after cutting.	Juice leakage and tissue softening. Translucency
Litchi (Lychee)	Bright red color, fruit size, Total soluble solids to Titratable acidity ratio (30:1- 40:1) (Underhill and Wong, 1990) depending on cultivar.	Peeling and de-stoning	α and β galactosidase	Degradation and solubilization of pectins	‚Juice leakage, tissue softening (Phanumong *et al*., 2015) Translucency,
Pear	Yellowish green color; >13% soluble solids	Peeling, coring and cutting into slices or cubes	Lipoxygenase, Phenylalanine lyase, PAL Polyphenol oxidase,PPO, pectinase	Lipid peroxidation, Synthesis of phenolic compounds PPO activity pectin degradation	Tissue Softening and cut surface browning, translucency

(Authors' own review: Adapted and modified from Kader, 2002 and Rahman, 2007 with modifications)

1.2.2 Effect of harvesting stress, time of harvest and climatic conditions

The earliest hours after harvesting of fresh produce are crucial for maintaining quality of produce for fresh-cut consumption.

Loss of quality in fresh-cut products can be attributed directly or indirectly to both abiotic stress and stress-induced senescence (Lester, 2003). Abiotic stress include extreme temperature, drought and other weather conditions like excessive rain during the time of harvest, whereas stress induced senescence may occur due to cracks, bruises and wounds as a consequent of harvesting method (manual or mechanical), excessive speed during transport and poor harvesting containers. This may lead to texture changes, loss of flavour and of important phytochemicals like Vitamin A, C, E and polyphenolics. Lester (2006) reported that carotene content of carrots increased when exposed to higher temperatures two weeks prior to harvest with a significant decrease in its typical orange color. On the other hand the quality of fruits and vegetables may improve in response to stress. Mechanical stress of lettuce by applying a 100 daily paper strokes, increased shelf-life by 33%, and this was associated with reduced plasticity and smaller leaf epidermal cells resulting in an enhanced ability to withstand washing activity of processing (Clarkson *et al.*, 2003). However, information on abiotic stress at the time of harvest is quite limited. This is because the effect of stress at this point may not be evident, but it is critical because, it contributes to shelf-life and appeal characteristics of the final product.

Optimum climatic conditions at harvest (harvest season) and even the time of the day at which raw material is harvested, influence the development of desired flavors, taste, texture and color of fresh and fresh-cut products. The best harvest time for minimal processing depends on the variety (Moccia *et al*., 1998), the type of crop and the changes in respiratory activity related to the season of harvest (Garrido *et al*., 2015). As such, Garrido *et al.* (2015), reported that during spring, baby spinach harvested early in the morning (08:00h) had a higher water content, firm texture, lower respiration rate and better visual quality after processing, compared to product harvested at 13:00h and 17:30h, while they did not find any

difference with the time of the day in winter. In fresh basil leaves, content of essential oil was influenced by cropping season and storage time, with no significant effect of harvesting hour. Shoots harvested in January had twice the essential oil content of shoots harvested in August (Silva *et al.,* 2005). Donetti (2011) found rapid loss of firmness of avocado mesocarp harvested late in the season compared to fruits harvested early in the season. This is due to minor activity of pectin methyl esterase in the early maturity stages, (Ng *et al.*, 2013) compared to the late harvested fruits. (O'Neill and York, 2003). As for 2 varieties of artichokes, which produces several buds over the season, Ricci *et al.* (2013). reported that harvest season was very critical for post-cutting performance, showing that for *'Violetto foggiano'* artichokes harvested in the coldest date (February) were more suitable for fresh-cut processing, compared to late and earlier harvest dates, in terms of color and browning. Also for '*Catanese*' variety, artichokes harvested from January to February showed higher appearance scores than artichokes harvested in March and in April. These results were related to harvest temperatures and to the rainfall days before harvest; generally the post-cutting quality was negatively affected by the increase of temperature and days of rainfall.

In addition the natural decline of plants at the end of production was hypothesized to be an important factor affecting the poor quality of fresh-cut artichokes in the last harvest dates. Moreover a correlation of the visual appearance with antioxidant activity was found. The same authors reported that total phenolic content and antioxidant activity were different among harvest dates, and for '*Violetto foggiano*' a significant polynomial trend, denoting 2 phases of antioxidant accumulation, from December to February and then from middle of March to May was observed.

In addition also other authors reported that minimally processed artichokes harvested during the spring season were more susceptible to browning than those from the winter season, although artichokes harvested in the winter season had higher phenolic content (Massignan *et al*., 2005).

Furthermore, studies by Charron *et al.* (2005) showed that activity of glucosinolate degradative enzyme myrosinase in ten cultivars of *Brassica oleracea* (fresh weight

basis) was significantly dependent on the season. They found that light may have affected myrosinase enzyme activity through modulation of ascorbic acid, which at higher concentrations leads to inhibition of myrosinase enzyme, therefore reducing glucosinolate degradation. It could be inferred that the level of exposure of raw material to light prior to harvest may have influenced the level of some functional properties (Yabuta *et al.*, 2007).

Produce harvested early in the season, mainly when temperature is low, seem to be more suitable for minimal processing, whereas variations in processing and storage quality of fresh produce related to time of the day may be due to length of exposure to sunlight. Optimum radiation and temperature during harvest time improved total antioxidant activity of minimal processed rocket salads, provided that it was packaged without cutting (Venneria *et al.*, 2012). Different harvest times in a day (morning, afternoon and evening) affected chlorophyll, sugar content and color of broccoli florets during four days storage at 20 ^{o}C. Hasperué *et al.* (2011) described that samples harvested at 08:00h, 13:00h and 18:00h had similar dark green color, hue values and chlorophyll content, but after 4 days of storage samples harvested at 08:00 h had more yellow florets compared to samples harvested in the evening (18:00h), which showed the highest chlorophyll content, lowest sugar degradation and color changes.

Light is the main energy source for photosynthetic biogenesis (Walters, 2005; Hu *et al.*, 2007); and therefore exposure to light for longer periods prior to harvest may improve resistance of chlorophyll to degradation in storage as well as sugar content which is a photosynthetic product. Clarkson *et al*. (2005) also explained that improved processability and extended shelf life of baby leaves harvested at the end of the day was attributable to an accumulation of sucrose during the photoperiod following photosynthesis, with the highest sucrose accumulation in rocket salad leaves (salad roquette leading to prolonged shelf life of 2 to 6 days compared to lollo rosso and red chard (1-2 days). In addition, end of day salads also showed higher plasticity and/or turgor pressure which improved their resistance to

processing stress. All these considerations, demonstrate that good eating quality and could be retained in minimally processed fruits and vegetable, if fresh produce is harvested at the right time and maturity for processing, under optimum climacteric conditions. Considering, the biochemical and physiological processes of fresh produce and the raw material diversity, an all-inclusive research approach is required to sustain and improve consumer satisfaction regarding fresh-cut products. Research in this area, in recent past has focused on varietal factors (Gorny *et al*., 2002; Cabezas *et al*., 2009; Cornacchia *et al*., 2011; Colantuono *et al*., 2012; Silveira *et al*., 2013) and pre-harvest factors or cultivation practices (Ferrante *et al*., 2003; Gonella *et al*., 2004; Scuderi *et al*., 2011; Selma *et al*., 2012; Piazzolla *et al*., 2012; Luna *et al*., 2012; Bonasia *et al*., 2013) with limited studies on their collective effect on product quality after cutting and minimally processing.

Molecular tools are currently been used to enhance flavor, texture and produce quality parameters of fresh produce by promotion of synthesis or suppressing certain enzymatic activities at harvest; however, these produce may not provide the same characteristics when cut, due to cell wall disintegration, moisture loss and mixes in cell wall fluids leading to browning, surface dryness and other nutritional losses. Of course, some pre-processing and post-processing treatment interventions have been proposed and used but future studies in molecular agriculture should consider the effect of these biotechnologies on post-cutting quality of raw material.

1.3 Postharvest handling of raw material on cut product quality

The risk of obtaining a fresh-cut product with poor quality characteristics or rapid deterioration is a real problem affecting the industry; produce may have a poor quality at harvest or may lose their quality during field handling, through transportation, pack house operations, cooling, shipment and storage. Fresh fruits and vegetables harvested with desirable characteristics for processing can be contaminated by spoilage micro-organisms during handling processes which may render them not completely safe for consumption, even if washed with disinfectant (Barta *et al*., 2006). In addition, nutritional, phytochemical and other properties of

fresh-cut products, like color, flavor, taste and texture are also affected by poor handling of the raw material. Nutritive compounds may be triggered to change through physiological processes during postharvest handling, which can result in hydrolysis, breakage of polysaccharides into sugars, oxidation of sugars to pyruvic acid (glycolytic cycle), and aerobic transformation of pyruvic acid and other organic acids into CO_2 and water (Krebs cycle) affecting also taste and sensorial quality (Taiz and Zeiger, 2002). An in depth understanding of the postharvest physiology of fresh fruits and vegetables during these processes may help control specific processes that are responsible for changes in constituents and quality (Bartz and Brecht, 2003).

Generally, approved postharvest handling which include the selection and use of appropriate packaging, relative humidity and temperature (FAO, 2009), and also takes into consideration physiological as well as morphological differences of various fruits and vegetables, like roots (e.g. carrots), stem (e.g. asparagus), leaf (rocket leaves), immature (cucumbers), fully mature (pumpkins) and ripe fruits (tomatoes, papaya, Figures), (El-Ramady *et al*., 2015) are known to aid in maintaining quality of raw material. However, the extent to which this usual distribution, low mechanical stress and cooling stress can affect chemical constituents of fruits and vegetables have not received adequate attention (Tomas-Barberan and Gil, 2008). Postharvest metabolism involves catabolic processes (pectin hydrolysis, protein turnover, pigment degradation) and anabolic reactions (synthesis of enzymes, polysaccharides and organic acids, other proteins and pigments) (Haard, 1997). Also, auxins, gibberellins, cytokinins, abscisins and ethylene are the common phytohormones which influence physiology of fresh fruits and vegetables after harvesting. Although most studies have focused on ethylene, there is evidence that a balance of more than one hormone, is responsible for phytohormone activities (Haard, 2015).

Maintaining produce cold chain is critical for the retention of sensorial properties, phytochemicals and control of enzyme activities, eating quality of fruits and

vegetables as well as minimal processed products. Fresh-cut vegetables in many parts of Europe including Italy, have been estimated to have a shelf-life of 6-7 days, meanwhile depending on species and resistance to postharvest handling, fresh-cut produce exceed two weeks of shelf-life in the United States. This is achieved mainly due to the maintenance of cold chain temperatures below 4^{o}C after harvesting, during processing, shipping and distribution (Florkowski *et al*, 2014). Low temperature and relative humidity management during transport and storage play a critical role in maintaining product quality by reducing respiration rate, ethylene production and sensitivity, transpiration and water loss in addition to the growth of pathogens (Mitchell, 1992). However, in most cases more than one physiological attribute maintains the overall acceptability of a fruit or vegetable during postharvest handling for its intended use. For instance, during transport and storage of fresh tissues, low temperature, high relative humidity and artificial atmosphere storage are applied to delay physiological processes and loss of quality, since low temperature alone could be limit product quality by chilling injury or moister loss. An imbalance between appropriate temperature and relative humidity and an incorrect packaging or storage atmosphere may cause losses of moisture and weight which can affect appearance and quality of cut products after processing. For example during handling of produce with high sensitivity to chilling injury or high CO_2, low temperature and high CO_2 conditions should be avoided, as they may also accelerate losses of vitamin C (Lee and Kader, 2000). Details of the postharvest produce management and cut product quality are described in the following paragraphs.

1.3.1 Influence of transportation of raw material on fresh-cut quality

Postharvest transport impact on taste, color, flavor, phytochemicals and cut product quality is complex, as metabolic and emission mechanisms may vary according to species, cultivar and maturity stage at harvest, phenotypic characteristics like cuticle thickness; environmental conditions and other postharvest operations. Globalization and the international trade and demand for exotic products, call for postharvest transportation practices that will enhance quality and reduce senescence

processes, during transit and distribution. The fresh-cut processing industry is driven by the ability to provide a variety of products which are fresh, convenient and healthy. However inadequate transport processes may weaken this effort. Typically tropical and subtropical produce like mango, pineapple, papaya, avocado, or litchi, are shipped for long distances to Europe, some parts of USA and Japan. However, some of these fruits are chilling sensitive or prone to disease, decay and early senescence, reducing their quality during transit up to the point of becoming no longer suitable for processing. Intra EU trades are also very important. According to De Cicco (2016), three EU countries accounted for more than two-thirds of intra EU exports of fruits and vegetables in terms of value (EUR 33.4 billion) in 2015. These were Spain (leader in all exports except tomatoes, carrots and apples) followed by the Netherlands (for tomatoes) and then Italy (which ranked first exports for grapes and apples). The shipments are sometimes characterized by mixed produce transport and may also include produce for fresh processing. However, the efficiency of mixed produce shipment is based on compatibility with requirements for temperature, relative humidity, production of odor and ethylene. Limited by difficulties in temperature incompatibility, holding produce at its lowest possible temperature has been recommended, to also reduce ethylene production (Vigneault *et al.*, 2009).

In spite of the low temperature transport, produce may be susceptible to ethylene induced ripening; this may cause cross tainting in produce like kiwifruit due to its sensitivity to trace levels of ethylene (UK, P&I CLUB, 2006). Moreover accumulation of CO2 during transport, would affect the taste of the produce and its post-cutting performance; Ben-Arie and Sonego (1985) reported lower soluble solid content with increased CO_2 levels. Effective ventilation and prevention from contact of exhaust fumes during loading may prevent this condition. Fresh produce are either processed at the source of production or shipped to regional processors for processing. Impacts from transitory movements (conveying to temporal storage facilities, shipping, air-freighting) sometimes caused by unexpected acceleration or deceleration leads to great dissipation of energy and consequent damage to fruits

(Vignealt *et al*., 2002). In an experiment by Seljasen *et al*. (2001), it was shown that shaking carrots, simulating postharvest handling, induced poor and bitter taste due to the accumulation of 6-methoxymellein, and to a low content of glucose, fructose, total sugars and of total and individual terpenes (α-pinene, *p*-cymene, limonene, γ-terpinene and caryophyllene). This process limits the use of such carrots in fresh-cut processing as additional cutting stress may increase bitterness and enhance the production of ethanol during MAP storage.

Transit time and other environmental conditions may impact on either the release of flavor volatiles or enhance the emission of spoilage volatiles and off-odor during cutting operations and post-cutting shelf life. Typically volatile compounds from packaging materials and shipping containers could migrate into fresh fruits and vegetables depending on thickness of the cuticle, and produce uncharacteristic flavors or off-odors. Flavor compounds are of major importance in the fresh-cut products markets as aroma is appreciated by consumers and linked to freshness of the product. However the extent of migration depends on contact area between produce and packaging materials, storage temperature, the contact time, food composition, concentration of migrant, polarity and solubility of polymeric packaging materials and aroma compounds (Brown and Williams 2003; Linssen *et al.* 2003). Polyethylene materials are known to absorb many desirable volatiles from food (Sajilata *et al.*, 2007). Recent development of technologies that help with early detection of micro-organisms/pathogens, undesirable odors and taste may aid in the improvement of transport packaging of fresh fruits and vegetables for the fresh-cut industry.

1.3.2 Influence of pre-cooling of raw material on processing quality

Pre-cooling of raw material which arrives at the fresh-cut processing industry prior to processing is critical for enhancing the shelf-life and microbial quality of the final product. This aids in reducing field heat, respiration and physiological activities that may hasten senescence during post processing handling operations. Produce cooled at best recommended temperatures maintain optimum shelf life.

Ideally temperatures close to 0º C have been recommended for optimum shelf-life of leafy vegetables (Cantwell *et al.*, 1998, Koukounaras *et al.*, 2007), while for fruits and fruit vegetables, chilling sensitivity should be taken into account (Sargent, 1998). Different cooling methods may be applied to fresh produce in the industry, however the suitability for a specific fruit or vegetable is important. Fresh fruits and vegetables pre-cooling methods are mainly room cooling (RC), forced air cooling (FAC), hydro cooling (HC), vacuum cooling (VC), ice-packing (IP) and top-icing cooling (TI). In artichokes for instance, Colelli and Calabrese (2009) reported that rapid cooling of harvested artichokes to 2 - 4 º C in autumn and spring is critical, recommending hydrocooling, forced air and vacuum cooling. In the case of leafy vegetables low temperature (0-2 ºC) cooling method may be appropriate as they are more prone to water loss from the leaf surface and can affect final product quality when minimal processed. Garrido *et al.* (2015) found that hydro-cooling increased water content and color of spinach leaves, decreased pseudomonas counts significantly and reduced respiration rate in modified atmosphere packages, suggesting suitability for leafy vegetables especially in the spring season.

On the other side, vacuum cooling may have some negative effects on raw material quality. It has been reported to cause about 1 % produce weight loss (mostly water) for each 6 º C of cooling (El-Ramady *et al.*, 2015); increase tissue damage in spinach (Garrido *et al.,* 2015) and increase the infiltration of *E. coli* O157:H7 into lettuce tissue by more than 90% as compared to the non-vacuum cooled product (Li *et al.* 2008). Despite the shortcomings of vacuum cooling, it may also present some benefits as slight loss of water from low RH may improve rigidity of leafy vegetables and increase resistance to processing stress.

For tropical fruits like durian and jackfruit, a pre-cooling temperature of 10 ºC for 24 hours is recommended before fresh-cut processing. (Latifah *et al.*, 2013). However for citrus, Defraeye *et al.* (2015) also reported that ambient storage has been successfully tested for some varieties. Standing to these considerations, further research into method specificities and produce requirements may be important for optimum application to fresh produce before minimal processing.

1.3.3 Influence of storage conditions before processing on product quality

Storage temperature, relative humidity, eventual treatments and storage time of fresh fruits and vegetables prior to processing influences the tissue metabolic responses to wounding and senescence. Raw material stored under suitable temperature and humidity conditions have delayed senescence when minimally processed. Ricci *et al.* (2013) studied the effect of temperature (0, 5 and 12 °C) and time of storage before cutting on post-cutting quality of 2 artichokes cultivars 'Catanese' and 'Violetto Foggiano', and found that for 'Catanese' storage temperature of 0 °C was beneficial in delaying browning of cut-surfaces but induced the occurrence of brown spots on the outer bracts which appeared after 7 days of storage before processing. Also cantaloupe melon stored at 10 ^{o}C with relative humidity (RH) of 90±5% for 3 weeks produced fresh-cut products which were stored for 19 days at 2^{o}C and 87% RH with no change in colour quality, but with a 30% firmness decrease (Munira, *et al.* 2013).

Mature green tomatoes cooled below 13 ^{o} C, resulted in poor color quality when ripe (DeEll, 2004), reducing cutting quality. Pineapples cooled to 3 ^{o} C have been found to develop endogenous brown spot (Saltveit, 2017), which will cause losses in the fresh-cut industry. Besides the temperature and RH balance, high carbohydrate content in root vegetables also contribute to extend they shelf-life as they function as storage polymers for respiration (Edelenbos, 2015). Nonetheless the high carbohydrate content does not withstand fresh-cut processing, mainly as a result of the processing activity. Studies by Klaiber *et al.* (2005), showed that the concentration of carbohydrates in carrots as mono and disaccharides are lost during fresh cutting and washing.

Other ambient storage conditions may also impact on the shelf life of minimally processed products. Fresh-cut lettuce obtained from intact heads stored under light at a temperature of 12°C, looked fresh after 17days of storage, but resulted in a poor quality life due to a decline in soluble sugars, chlorophyll and an increase in electrolyte leakage of the intact tissue (Witkowska and Woltering, 2014).

Storage treatments like heat-treatment, 1-MCP treatment and modified atmosphere may also impact on cut product quality, as described in the following paragraphs.

1.3.3.1 Heat treatments

The use of heat treatments has been introduced as an additional technology combined to cold storage to reduce chilling injury, delay ripening processes, control pathogen and disease as well as insect control (Paull and Chen, 2000; Tang *et al.* 2007) of fresh produce during storage and therefore may have also a direct effect on quality and storability of cut products. Particularly different heat treatments applied before cutting have been tested to increase shelf-life of cut products, like wedged shaped peaches (Obando *et al*., 2010) kiwifruit slices (Beirao-da-Costa *et al*., 2006); melon (Lamikanra and Watson, 2007); mango (Djiouna *et al*., 2009); whereas some studies also refer to the application of heat treatments after cutting, as in green onions (Hong *et al*., 2000).

Heat treatment mechanism contributing to enhance quality and shelf-life of fresh-produce was described by Wang *et al*. (2001) as an induced increase in transcripts heat-shock proteins (molecular weight of 17 and a 70 KD) which reduces chromatin condensation and DNA breakdown, and suppresses oxidative activity. Heat treatment also delay firmness losses by inhibiting the solubilization of carbon soluble pectin fractions, (Shalom *et al*., 1996). Heat treatments vary from curing, hot-water dipping, vapor heat/water-saturated air, hot dry air, hot water rinsing and hot brushing and have been proposed on produce like onions, mango, avocado and spinaches (Schirra *et al*., 2000; Schroeder and Du Toit, 2010; Fallik, 2004; Weller *et al*., 1999; Glowacz *et al*., 2013) Other quite recent heat treatments include, electromagnetic radiation (microwave and radio frequency energy) and far infrared heating (Geysen, 2005).Treatment temperature for whole produce ranges from 30 °C to 40 °C in hot air and can be applied for several hours up to days, whereas water dipping at the temperature of 50–56 °C are applied for few minutes, and higher temperatures up to 63 °C in hot water rinsing and brushing for 10-25 seconds (Lurie, 1998; Fallik, 2004).

Studies by Artes *et al.* (2000) showed that intermittent warming of pomegranate whole fruit, stored at 2^{o} C yielded arils with the better flavor, higher anthocyanin content and higher storability (13weeks) compared to curing which resulted in low flavor quality of arils. Temporal rise in temperature may induce higher metabolic activity, which allows tissues to produce excessintermediates which may help to repair cold damaged membrane and metabolic pathways (Lyons and Breidenbach, 1987; Wang, 2010). However, such excess intermediates may include non-enzymatic antioxidant or low molecular weight compounds like anthocyanins and other polyphenol pigments, which unfortunately may affect visual quality of some fruits when used as fresh-cut, even though other quality parameters are maintained. For example, Murray *et al.* (2007), found out that heat treatment of 'Flavortop' peach at 39^{o} C for 24 hours, improved juiciness, commercial quality and delayed internal breakdown for 4 weeks, except for the increased the red color of the peel and the flesh. However the authors recommended post processing storage in controlled atmosphere as a preventive option of this effect. Hot air treatment of MD2 variety of pineapple at 73.49 ^{o}C for 10 minutes before cutting reduced loss of vitamin C and total soluble solids, and produced golden brown, firm and sweet-sour balanced cut products with refrigerated shelf life of seven days (Angba, 2010). This mechanism presents an opportunity for improving raw material quality for fresh-cut product due to its ability to minimize oxidative damage which results from cutting, peeling and shredding activities.

1.3.3.2 Ozone treatment

Ozone treatment of fruits and vegetables during storage has been accepted in the industry due to its non-residual effect on fresh produce after treatment. Its use in the postharvest industry is increasing (Parish *et al.,* 2003), as the technique extends the shelf life of stored raw fruits and vegetables (Abdel-Wahhab *et al.*, 2011). Currently ozonized cold storage is being extended to the fresh-cut processing industry, where produce are stored and pre-cooled in ozonized rooms prior to processing (Personal communication). However the effectiveness and sustainability of this approach depends on the concentration, the type of fresh produce and the duration of

exposure to the gaseous ozone. In a study on papaya, freshly harvested fruits were exposed continuously to various ozone fumigation (0, 1.5, 2.5, 3.5 and 5ppm) for 4 days prior to an ambient storage at 25 °C and 70 % RH for up to 14days. Fruits exposed to 2.5ppm ozone had higher levels of total soluble solids, ascorbic acid, β-carotene, lycopene content and antioxidant activity, reduced weight loss and overall quality acceptability at day 10 compared to the control (Ali *et al*., 2014). In a study on baby leaves, samples stored under 2 ppm ozone lost their fresh appearance irrespective of storage temperatures of 4^{o}C and 10^{o}C whereas in samples stored at 0.5ppm, leaves maintained the same quality as untreated samples (de Candia *et al.,* 2015). In another study discolorations of lettuce was observed due to long term exposure (10 and 15 mins) and higher concentrations of 5.2mg/L and 7.6mg/L (Singh *et al.,* 2002).

Use of the right dosage and treatment procedure may present benefits for storage of raw material prior to processing. A humidified flow of ozone enriched air applied cyclically (4 ± 0.5 μL L^{-1} of O_3 for 30 min every 3 h) at 5^{o}C produced firmer tomatoes (cv 'Thomas') with higher sugar and organic acid content despite initial increase in respiration rate (Aguayo *et al.*, 2006), thus improving raw material quality for fresh-cut processing. The same authors applying ozone on sliced tomatoes of the same variety reported that although slices kept good appearance quality, their aroma was compromised., These results suggested that whole produce treatment prior minimal processing may be a better option, due to the minimal effect of ozone on the internal tissues resulting from its inability to permeate the skin surface of fruits (Skog and Chu, 2001). Phytochemical properties and color pigments like ascorbic acid (Pérez *et al*., 1999) and anthocyanins in strawberry (Keutgen and Pawelzik, 2005), chlorophyll content in parsley (Karaca and Velioglu, 2014), lycopene content in tomatoes (Aguayo *et al*., 2006) have been retained after exposure to ozone treatments.

Skog and Chu (2001), observed that ozone gas could be used in controlling ethylene in storage rooms and suggested the possibility of storing ethylene-producing apples and pears with ethylene sensitive broccoli in the sameroom provided ozone is used. This could be applicable in the fresh-cut industry where produce are temporary stored prior to processing. One limitation of ozone treatment is its inability to penetrate cuticular folds and minute cracks on leafy vegetables (Babic *et al*., 1996). However, Vurma *et al*. (2009) suggested that the diffusion of gaseous sanitizers like ozone into leafy green produce can be improved by first subjecting the product to vacuum cooling prior to the gas treatment, since interstitial spaces can ease the gas diffusion into the product.

1.3.3.3 1-Methylcyclopropene (1-MCP) treatment

The use of 1-MCP for storage or for long-distance transport seems promising (Sivakumar *et al.* 2011), however it effects on the subsequent cut product has not been well explored. The application of 1-MCP treatment of Keith mangoes (at 1µL/L). alone or as a pretreatment before hot water quarantine, has been suggested as a method for reducing softening and peel color changes of fruits (Ngamchuachit *et al*. 2014).. Manenoi *et al*. (2007), reported that though 1-MCP treated papaya fruits at the color break stage (25% yellow) delayed softening and disease incidence (50-1000nl/L), it produced 'rubbery' fruit pieces possibly due to low recovery of ethylene biosynthesis. This may impact on texture quality and palatability when used as a cut product. On the other hand, for apples, the application of 1-MCP (600 nl/L) before cutting, gave firmer fresh-cut product without any other quality (color, SSC, TA and total phenol content) differences compared with non-treated fruit (Georgoudaki and Nanos, 2014).

In another study on 'Fuji' apples, treatment with 1 µL/L 1-MCP for 10 h at 20℃ prior to cutting significantly reduced wound-active responses including respiration of fresh-cut product (Mao *et al*., 2007). Also for tomatoes, exposure of 1 µL/L 1-MCP to intact fruits for 12 hours at 20 ^{o}C was effective in inhibiting ethylene-induced softening in its sliced products (Pangaribuan and Irving, 2010). However,

Cornacchia *et al*. (2007) reported that exposure of whole tomato fruits to 1-MCP before cutting induced firnmess losses in comparison to the application of 1-MCP after cutting which resulted in a lower production of ethylene of the slices. For kiwifruits slices, treatment of whole fruit with 1-MCP application at 1μL L^{-1} for 6 hours at 10ºC delayed rate of softening and ethylene production (Vilas-Boaz and Kader, 2007). In leafy vegetables, fully expanded rocket leaves (**Eruca sativa** Mill.) treated with 1-MCP at a concentration of 0.5 μl L^{-1} for 4 h at 10 °C before storage for 10 days in air or in air with ethylene (1 μl L^{-1}) prevented yellowing of leaves, increasing their shelf life (Koukounaras *et al*., 2006). Also, Broccoli raab florets treated with 1 μL L^{-1} of 1-MCP for 24 h at 20 °C and stord at 5 °C for 14 days in a humidified air flow showed an extended the shelf life, reduced chlorophyll degradation, and delayed loss of visual quality loss even in the presence of exogenous ethylene compared to untreated samples (Cefola *et al*., 2010). From these results it may be concluded that 1-MCP has been effective in reducing mainly ethylene and wound-active responses like increase in respiration and quality loss in fresh-cut products, but it is noteworthy that while inhibition of ethylene production may decrease storage disorders related to chilling injury as in avocados and pineapples; it may also increase chilling-related internal breakdown of peaches and nectarines (Huber, 2008; Watkins, 2008; Mahajan *et al*., 2014) and hence limit their use for fresh-cut processing.

In addition, the type of formulation used on whole fruits or vegetables may negatively impact on the cut product quality. Typically, aqueous 1-MCP, shows similar effects as gaseous 1-MCP, but in produce like avocado, cell wall-associated enzymes, antioxidant and volatiles are delayed in recovering, and reach levels similar to those of untreated fruits when the aqueous form is used (Zhang *et al*., 2013). It is therefore important that suitability of application for whole produce and its fresh-cut counterpart is further assessed. Particularly, the maturity stage of application, the concentration, the form of application (e.g. aqueous or gaseous) and time of exposure, allowing to recover regular biosynthesis activity, preserving flavor, taste, color and phytochemicals of fresh-cut products, need further research.

1.3.3.4 Controlled and modified atmosphere storage

The influence of controlled and modified atmosphere storage on shelf life of whole and cut products is well- known. This storage approach reduces metabolic activities of raw material in storage and may be applied on whole product to influence the post-cutting quality as well. Few studies addressed directly this aspect, whereas references of optimal atmosphere to store whole produce (Yahia, 2009; Mangaraj and Goswami, 2009; Kader, 2009; Sharma *et al*., 2011; Ramayya *et al*., 2011) and fresh-cut produce of sliced zucchini, shredded iceberg lettuce and onions (Gorny, 2003); carrots (Simões *et al*., 2011); Kiwifruit (Cornacchia *et al*., 2008); Pineapple (Finnegan *et al*., 2013) may be easily found.

Abreu *et al*. (2012), found that whole Exposition of 'Rocha' pears to 100% O_2 (superatmospheric oxygen) at 5 ºC for 30days was effective in delaying pericarp browning and sensorial losses of fresh-cut fruits, which showed a 7-day shelf-life extension compared to control samples. Also cut Bartlett pears previously held at -1°C in a controlled atmosphere with 2% O_2 + 98% N_2 had a longer shelf- life than those obtained from fruits held in air (Gorny *et al*., 2000). Despite cultivar characteristics, apples previously stored in elevated CO_2-atmospheres with diphenylamine produced slices with lighter fruit color than fruits stored in air, however stored fruits produced softer slices than those at harvest (Amissah *et al*., 2006). The authors also recommended that maintaining whole apple fruit quality by CA will produce slices with the best quality and longest shelf life.

Storage temperature, relative humidity, length of storage and pre-treatments of fresh fruits and vegetables prior to processing influences the quality of minimally processed or cut product.

1.4 Conclusion and Future Prospects

Physical damages, physiological and biochemical changes during harvest and postharvest have always been a limitation to enhancing diffusion of fresh-cut product on the market. Still, consumers demand products with uniform taste, flavor, texture and visual quality year round. This imposes a burden on the fresh-cut processing industry as its needs to meet customer interests to remain in business. This review paper has provided some highlights on harvest and postharvest handling activities that influence quality of certain types of whole produce and their possible impact on fresh-cut products. Optimization of factors like harvesting systems, time of harvest and maturity stage for produce intended for fresh-cut processing is critical for addressing quality issues and sustainability of the industry. Molecular research should consider resistance of raw material to postharvest handling, storage and fresh-cut processing during varietal or cultivar development. Despite the fact that maintaining the cold chain is important, care should be taken in postharvest handling activities like transportation, storage, and application of postharvest treatments (like ozone, heat treatment, 1-MCP, CA and MAP storage) before processing. Physiological, biochemical, and enzymatic reactions occurring before cutting may in fact, influence the final quality and shelf-life of the fresh-cut products.

2.0 Review paper 2: Effects of minimal processing activities and equipment on the quality of fresh-cut products

2.1 General Introduction

A variety of fresh conveniently packaged, minimally processed products also known as lightly processed or fresh-cut products are available on the market in response to consumer demand for ready to eat food worldwide. Majority of these products are leafy vegetables which are highly susceptible to quality changes during minimal processing activities (trimming, cutting, washing, centrifugation or drying of surface water) which affect their sensory and nutritional attributes. The sensory attributes are mainly appearance (color, freedom of defects); texture (firmness, crispness, juiciness and toughness based on the commodity); taste and flavor (sweetness, sourness, acidity, astringency, aroma, off-flavours) while the nutritional attributes comprises of the content of vitamins, minerals, antioxidant phytonutrients (phenol compounds, flavonoids, chlorophyll and carotenoids) and other bioactive components with curative and preventive effects on some health diseases (Kader 2001; Kader and Barrett 2005; Brecht *et al*., 2004; Artes & Allende, 2005; Rico *et al*., 2007).

Quality changes occur as a direct effect of wounding during minimal processing and hastens product deterioration processes through a number biochemical and physiological changes affecting the viability and quality of the produce (Brecht, 1995; Saltveit, 1997, 2016). These effects are initiated by the production, transmittance and perception of unique wound signals in the product (Cisneros-Zevallos *et al*., 2014, Toivonen and DeEll, 2002, Saltveit, 2003) when the protective membrane is injured; and contributes to water loss, cell-wall degradation and loss of firmness, discoloration, loss of flavor, microbial entry and spoilage, which are mainly influenced by temperature and handling processes.. An example is loss of ascorbic acid in fresh-cut kiwifruit at higher temperatures while in cucumbers, no reduction in ascorbic acid occurred at 20 ^{o}C but rather at 5^{o}C (Agar *et al*., 1999; Lee and Kader, 2000). Processing and handling of fresh produce at appropriate low temperature, relative humidity, optimum atmosphere storage and

suitable packaging protects their color, texture, flavor and nutritional attributes (Kader, 2002; Paull, 1999).

Despite the available precautionary measures for maintaining quality attributes as mentioned, alteration of physiological processes of the produce during minimal processing is unavoidable. However, the extent to which quality is compromised depends on the produce and the processing environment (processing activities and the equipment used). Produce characteristics include the type of crop (tissues, organs and its composition), respiration rate, time of harvest, maturity stage and any pre-processing treatment it may have been subjected to, prior to processing. The processing environment include, the temperature in the facility, water quality, sanitizer used, the equipment used during processing activities, including packaging requirements. Therefore, understanding the changes that occur during minimal processing (cutting, chopping, shredding, washing and drying) and how each processing activity and equipment used contribute to product stress and quality losses will aid in improving minimal processing and product quality.

Physical, physiological and biochemical changes, microbial entry and chemical contamination are the main factors affecting minimal processing quality, caused by the minimal processing operations, technological and type of equipment used. This reviews seeks to assess these changes in order to identify areas for future research aiming at the enhancement of product quality.

2.2 Physical, physiology and biochemical effects of minimal processing

The physiology of minimally processed leafy vegetables, comprises of respiration, transpiration, hormonal imbalance and ethylene production as well as enzymatic activities related to oxidation, loss of pigments and discoloration. All fresh-cut products have accelerated metabolism due to cutting, washing and other operations that affects their quality, and therefore there is a huge difference between fresh-cut products and whole produce in terms of physiology and handling requirement. That notwithstanding, the physiology of the minimally processed or cut leafy vegetables

is the same as that of the wounded intact product. The response of fresh produce tissue to wound signals occur within seconds, minutes and hours from the damage. Within seconds from wounding, in fact, a signal is generated then transmitted to adjacent tissues, inducing numerous responses (Saltveit, 2015).

This is followed by an accelerated metabolism, accompanied by increased respiration and ethylene production within minutes, (Figure 2.1) (Saltviet, 2003, Cantwell and Suslow, 1999). This metabolic and respiratory effect leads to increases in biochemical and enzymatic reactions that causes discoloration, browning, tissue softening and membrane breakdown (Toivonen and Brummell, 2008; Hodges *et al*., 2000); that decreases nutritional components like sugars, organic acids and vitamin content (Cantwell and Suslow, 1999, Gil *et al*., 2006) and causes changes in aroma volatiles as well as off-odor development (Beaulieu, 2006). Depending on the duration and intensity of the processing operations, degradative changes in the final minimal processed product can vary in extent. However, packaging in modified atmosphere immediately after processing will aid in minimizing quality loss. On the other hand, wounded vegetable tissues may go through wound healing process to achieve stasis again, but this process is inhibited by the use of the low temperatures (5 ^{o} C) applied for processing and storing minimal processed products. Fugate *et al*. (2016) reported delays in wound healing and reduced lignin and suberin accumulation at 6 ^{o} C compared to storage at 12 ^{o} C. However, synthesis of lignin may be undesirable in products like asparagus, which is associated to an increase of fiber and to poor eating quality. These changes are illustrated in the Figure 2.1.

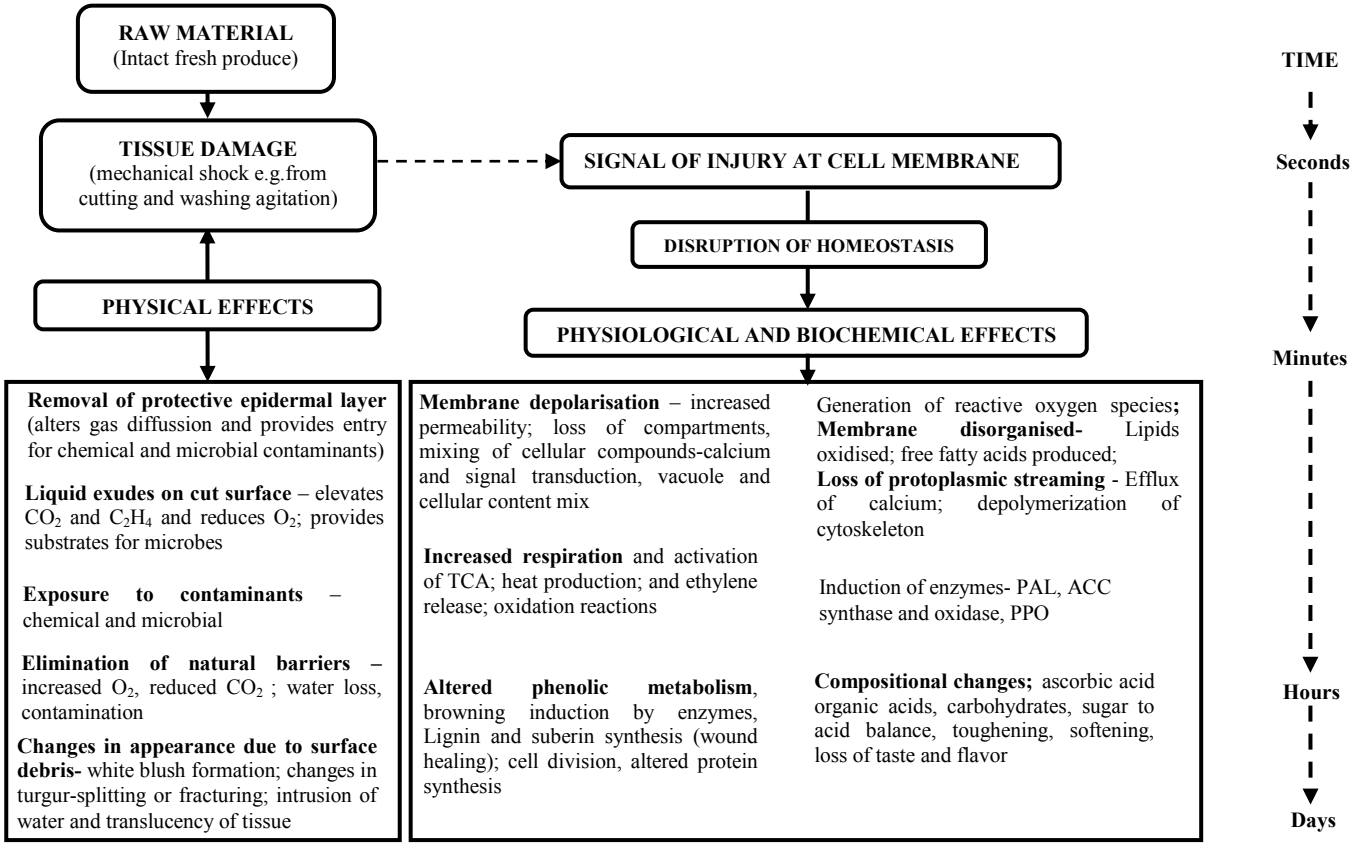


Figure 2.1: Physical, physiological and biochemical changes in cells and tissues after injury/wounding during minimal processing of leafy vegetables – Time scale is not absolute (Adapted from Saltveit, 1997, 2003; Muller, 2002)

2.2.1 Physical Effects

2.2.1.1 Loss of Epidermal Layer and changes in gas diffusion

Minimal processing activities, mainly mechanical processes like vibration and agitation from washing activities of baby or adult leaves; tearing, cutting or shredding of whole product causes bruises and breakage of product surface tissues. As a consequence, water starts to exude from the damaged tissue cells onto the surface of the product, inhibiting gas diffusion (gas moves through air 10,000 times faster than through water) and causing an increase in carbon dioxide and a decrease in oxygen levels in plant organs altering metabolic processes (Saltveit, 2003). This may even be worsened by washing processes which adds more water onto product surface and may further decrease oxygen levels if product surface are not well dried during centrifugation or air- tunnel drying activities prior to packaging. Internal O_2 concentrations below 5 kPa, causes partial inhibition of respiration, decrease in the cellular energy status, and partial inhibition of other energy-consuming processes (Geigenberger *et al*., 2000). This leads to fermentation, microbial contamination and other physiological effects which are described in subsequent sections.

2.2.1.2 Loss of Epidermal Layer and Water Loss

Wounding from minimal processing activities removes the natural barriers to water loss and exposes water of intercellular spaces which are otherwise enclosed to the outside atmosphere. Typical activities like cutting exposes the hydrated interior tissues of fresh produce and significantly increases the rate of respiration (Brecht *et al*., 2004). According to Burton (1982), the degree of water loss between intact and wounded tissue varies based on the surface characteristics of the product. For lightly suberized organ surfaces like that of carrot and parsnip, 5-10 fold of water loss occurs; for organs with cuticularized surfaces like spinach leaf and cucumbers, 10-100 fold and for highly suberized surfaces as in potato tubers up to 500 fold. The rate of water loss or transfer from the vacuoles of the damaged cells depends on the severity of the wound and surface area. The high the surface to volume ratios, the more water will be lost from the product. Desiccation from water loss causes

wilting, surface dryness and poor appearance quality (e.g. white blush in carrots), loss of crispness and texture changes and translucency of minimal processed leafy vegetables. Appropriate packaging can retard water loss in minimal processing products (Toivonen and Brumell, 2008), in addition to suitable temperature conditions and relative humidity.

2.2.2 Physiological and Biochemical Effects

2.2.2.1. Respiration and Metabolism

Respiration of leafy vegetables continues after harvest. The rate of respiration in minimal processed products is influenced by physical or mechanical stresses, maturity stage, and temperature and atmosphere composition. It involves the breakdown of cellular substrates like sugars, starch and organic acids which affects product weight, turgor, sweetness and flavour (Silva, 2008). The main processes involved in aerobic respiration metabolic pathways are glycolysis, the tricarboxylic cycle (TCA) or Krebs cycle and the electron transport system (Kader and Saltveit, 2003; Wills *et al*., 2007).

Under severe stress conditions, as for example after cutting, respiration rate increases. Cutting wounds on produce tissues increases respiration and ethylene production (Artes-Hernandez *et al*., 2013). Increasing the number of cutting increases respiration rate. As observed by Cantwell and Suslow (1999), cutting iceberg and romaine lettuce (2-3 x 2-3cm), increased respiration rate by 20-40% compared to their intact heads, while severe cutting of lettuce and cabbage into shreds resulted in increased respiration of 200 -300%. In other studies, shredded cabbage at temperatures of 7.5^{o} C and 10^{o} C, resulted in increased respiration by 2-3 folds (Cantwell, 1992; Toivonen and DeEll, 2002) compared to the whole product. Such high respiratory activity results in the depletion of respiratory substrates leading to the use of fats and proteins in the form of fatty acid, amino acids and glycerol and producing C6- and phenolic volatiles like phenyl aldehyde and benzaldehyde (Myung *et al*., 2006; Rambla *et al*., 2014).

Loss of chlorophyll in green vegetables is another consequence of increased respiration (Brar *et al*., 2013); in some cases loss of chlorophyll leads to browning, it occurs when the chlorophyll bound magnesium atom is removed or lost and substituted by hydrogen to form pheophytin, an olive coloured pigment. Colour changes from bright green to olive brown can only be observed when more than 50% conversion of the chlorophyll to pheophytin has occurred (Lau *et al*., 2000). Respiratory activities also leads to transpiration, which may induce dehydration, wilting, shrivelling, loss of juiciness and crispness affecting texture and appearance of leafy vegetables, if not well managed during minimal processing. Losses of total chlorophyll, soluble sugars, starch, and soluble amino acids has been reported to occur immediately after minimal processing of vegetables (Simões *et al*., 2010). Leafy vegetables are mainly non-climacteric and hence produce relatively low levels of ethylene to cause senescence under stress conditions. Though young leaves may have higher levels of ethylene than older leaves, its concentration in the plant tissues does not promote yellowing. Koukounaras *et al*. (2006; 2007) reported that, yellowing in rocket leaves as consequence of chlorophyll degradation was more related to exogenous ethylene than to the production of endogenous ethylene. Loss of green colour in leafy vegetables during minimal processing may be more related to chlorophyll leakage (Simões *et al*.,2010), since the reduced time period needed for preparation may not allow chlorophyll degradative enzyme activity to occur.

2.2.2.2. Membrane Stability and Lipid degradation

The membrane is a selective barrier composed of phospholipids (a bilayer of proteins within a lipid matrix), that provides compartment for substrates, enzymes, metabolites, molecules and mineral elements for normal metabolic activities (Taiz and Zieger, 2006; Antonacci *et al*., 2011). A few hours after wounding (cutting, manipulation and agitation from washing), activated phospholipase enzymes D (PLD, EC: 3.1.4.4) and A2 (PLA2, EC: 3.1.1.4) breakdown the phospholipids in the membranes accumulating phosphatidic acid (PA), phosphatidic alcohols and unesterified fatty acids (Wang, 2000; Narvez-Vasquez *et al*., 1999; Saltveit *et al*.,

2005; Ryu and Wang, 1996). The release of PA from the PLD enzyme activity is recognized as the first reaction in the membrane degradation catabolic pathway regulating further sequence of enzyme reactions and the flow of metabolites (Creelman and Mullet, 1997; Leon *et al*., 2001; Bhushan *et al*., 2015). PA is converted to diacylglycerol (DAG) by phosphatidate phosphatase (Katagiri *et al*. 2005), and DAG is converted to linolenic and linoleic acids (LA)-free fatty acid by a lipolytic acyl hydrolase (Ryu and Wang 1998).

The release of free fatty acids serve as substrates for lipoxygenase (LOX, EC: 1.13.11.12) enzyme, which catalyses the formation of conjugated hydroperoxides, resulting in the generation of free radicals that further disrupts membranes, leading to the generation of desirable and undesirable aroma volatiles (de Bruxelles and Roberts, 2001; Mazliak, 1983).

Research by Saltveit *et al*. (2005) shows that though phosphatidic alcohols are thought to be metabolically inert, they may also play a role in reduce wound induced stress in leafy vegetable. Further research on this topic may aid in improving fresh-cut product quality.

Metabolic activities increasing due to the mixing of intracellular and intercellular enzymes and substrates also contribute to textural changes during and after processing (Beaulieu and Gorny, 2003). Pectinolytic and proteolytic enzymes for example PG and α- (EC 3.21.22) and β-galactosidase activity exuding from injured cells due to the cutting activity diffuse into inner cells causing loss of firmness (Karakurt and Huber, 2002; 2003).

Membrane degradation is therefore one of the key factors for quality losses in leafy vegetables since it is the primary cause for texture, colour and aroma changes both on the surface of the product and at the cellular level.

Although some work has been done on the PLD enzyme activity in fresh-cut products, the emphasis has been put on the cutting activity, storage treatment or storage quality and most often on fruits (Saltveit *et al*., 2005; Mao *et al*., 2004; Karakurt and Huber, 2003). However, considering the principal role that PLD and PA play in the initiation of membrane degradation, they could be used as biomarkers in improving cutting operations, washing procedure, and even the appropriate holding time of minimal processed products prior to packaging for enhanced shelf life and quality of final products. Holding time as used refers to the time in which minimal processed product that is produce cut, washed and dried, are held prior packaging.

2.2.2.3. Wound Healing

Wound healing effect on minimal processed vegetables products has not received much attention, however, injuries to minimal processed products may induce stress responses or wound healing process which may incur positive or detrimental effects on the product. For instance, the injury or wound-stimulated phenylpropanoid metabolism promotes synthesis and accumulation of phenolic compounds that promotes browning of fresh-cut lettuce (Saltveit, 2000). On the other hand, an increase in tissue electrolyte leakage from cutting cilantro decreased during early storage (Luo *et al*., 2003), possibly due to the initiation of wound healing and membrane recovery process (Ramamurthy *et al*., 2000). The wound healing process in minimally processed vegetables may lead reduced microbial entry and growth (Watada *et al*., 2005), however cold storage conditions may delay wound healing process. Probably temporal temperature stress after minimal processing prior to storage may enhance the benefits of the wound healing process on product quality. Care should be taken in products like carrots, where wound healing and lignification causes a negative effect known as white blush (a white covering on the cut surface of carrot due to dehydration), within the first 2 hours of processing with or without packaging at 5^{o} C (Simões *et al*., 2010).

2.3 Impact physical and physiological effects on quality changes

2.3.1. Changes in phytonutrients

Leafy vegetables are rich in phytonutrients important for many biological processes. These phytonutrients include vitamin C, carotenoids, flavonoids, chlorophyll, polyphenols, glucosinolates, phytosterols, folate, potassium, magnesium, dietary fibre which contribute to antioxidant and antibacterial activity, stimulate the immune system or modulate enzyme activity (Lampe, 1999; Kris-Etherton *et al*., 2002; Hall *et al*., 2012; Martínez-Sánchez *et al*., 2008a). Besides the nutritional benefits, biochemical changes that affect the phytonutrients influences the visual quality of vegetables and may indicate freshness or deterioration.

During minimal processing phenolic metabolism increases and causes an accumulation of phenolic metabolites in fruits and vegetables (Tomás-Barberán *et al*, 2000). Cutting of vegetables causes an increase in wound-induced phenolic levels which contributes to improving total antioxidant capacity of the product (Reyes *et al*., 2007). However, wounding affect the concentrations of other nutritional compounds like ascorbic acid, beta-carotene and flavonoids (Yadav & Sehgal, 1995; DuPont *et al*., 2000) which can be oxidized as consequence of the stress and damages related to processing. High ascorbate activity is induced due to increased respiration and wounding, which immediately transforms ascorbic acid to dehydroascorbic acid (Gil *et al*., 1998). Hence the ratio of dehydroascorbic/ascorbic acid is considerably higher in minimal processed vegetables (Buescher *et al*., 1999). Although ascorbic acid and dehydroascorbic acid, make up total vitamin C content with antioxidant properties, ascorbic acid has a much higher reducing capacity and antioxidant potential than dehydroascorbic acid (Davey *et al*., 2000). However increase of phenolic compounds may counteract the losses in ascorbic acid and maintain the antioxidant status of leaves, inhibiting any associated postharvest disorder (Ferrante *et al*., 2009).

In the case of glucosinolates, their molecular structure changes when the cell is damaged (Kim and Jander, 2007); which may also result from processing activities. The cutting, vibrating and shaking processes may cause glucosinolate in vacuole to mix with myrosinase in the cytosol, leading to the formation of metabolites. This reaction causes a release of glucose, sulfate and several hydrolysis products of glucosinolates, including epithionitrile, thiocyanates, nitriles, isothiocyanates, oxazolidine-thione and other minor compounds (Rask *et al*. 2000). Glucosinolate content in minimally processed products can also be linked to the stage at harvest. In rocket leaves, subsequent cuts after the first harvest have reduced glucosinolate content due to disruption in normal metabolism after each cutting activity (Hall *et al*., 2015).

Carotenoids are quite stable compounds. However due to the double bonds in their carbon chain, they may be prone to reactions like oxidation and isomeratisation (cis-trans) during processing, especially when exposed to oxygen, light and acids which may cause loss of colour and a reduction in their bioactivity (Rao and Rao, 2007; Rodriguez-Amaya, 1999). Oxidation of carotenoids is due to oxygen exposure, catalysed by enzymes such as lipoxygenase. In addition, low moisture levels during processing of vegetables may cause losses of up to 50% in the concentration of carotenoids (Dorantes-Alvarez and Chiralt, 2000). However minimal processing activities does not result in significant changes in carotenoid content, thus both fresh and minimal processed leafy vegetables are likely to have the same levels of β-carotene (de Azevedo and Rodriguez-Amaya, 2005);, though post processing storage may cause changes.

2.3.2 Changes in Sensorial properties

Colour is the main attribute contributing to the visual appealing properties of minimal processed product. It provide information on the freshness of a product or any changes or deterioration occurring in the product through instrumental measurements and visual inspection. Physical damages from minimal processing activities and their subsequent physiological and biochemical processes, may cause

colour changes reducing the quality of the product. Membrane degradation resulting in mixing of enzymes and substrates may lead to browning, since red or dark pigments on cut product surfaces are formed making the product unattractive. This brown change in colour on product surface (for example pink discoloration in iceberg lettuce; dark discoloration in potato or artichokes) occur through oxidative reactions of phenolic compounds by polyphenol oxidase, which produce o-quinones reacting non-enzymatically to produce polymerized products called melanins (Tomas-Barberan and Espin, 2001; Soliva-Fortunry and Martin-Belloso, 2003). Bright green colour of leaf vegetables are associated with high chlorophyll content and freshness, however physiological process like respiration, as explained previously, may lead to yellowing in products like rocket leaves and chicory, also associated with carotenoid degradation (Ferrante *et al.*, 2004). In products like cabbage loss of chlorophyll and green colour may lead to lighter white colour as they do not have pre-existing carotenoid pigments to turn yellow (Heaton *et al*., 1996).

Texture properties of minimally processed products depend on the cell size and turgor pressure, thickness of the cell wall and the closeness with which the cells are held together (Harker *et al*., 1997). Main texture attributes are firmness, crispiness, juiciness, toughness, and softness and depend on the type of product. During minimal processing water loss or water exuding from damaged cells onto product surfaces due to cutting, causes tissue and cell wall disintegration, and remove turgor pressure causing dryness and loss of firmness, as reported in bell pepper (Cantwell *et al*., 2001); or softening from activated cell-wall degradation enzymes (Watada *et al*., 2005). Regarding the physiological response and senescence related enzymatic changes, toughening can also occur as a result of lignification of tissues (Smith *et al*. 2002).

Aroma volatile compounds play a critical function in improving sensory characteristics of leafy vegetables due to their contribution to flavour and taste. However, minimal processing can lead to alteration of flavour active aroma profile of vegetables tissues compared to their intact products. Typically, cutting and handling of vegetables during minimal processing lead to an unquenchable process of release of volatiles. This occurs through enzymatic degradation resulting from the mixing of enzymes with primary and secondary metabolites (phytonutrients), that are otherwise separated in the intact tissue, or by oxidation or autoxidation reactions (Husain, 2010). Fatty acids/lipids, amino acids, and carbohydrates are among the primary metabolites, whereas carotenoids, glucosinolates, phenolic and terpenoids are the secondary metabolites generating volatiles (Buttery and Ling, 1993; Schwab *et al*., 2008).

Fatty acid degradation is responsible for the fresh green fruity aroma notes of vegetables. It occurs in three different oxidative routes. One is the β-oxidation pathway which may not be relevant in minimal processing as it occurs in intact produce. The other two are the oxidation by the lipoxygenase (LOX) and the autoxidation pathways which may occur during minimal processing and post processing storage.

The LOX pathway is composed of enzymatic proteins that oxidize free polyunsaturated fatty acids, including linoleic, linolenic acids to produce C6- and C9- aldehydes (Matsui *et al*., 2006). Upon wounding or tissue disruption, the fatty acids increase as consequence of cellular events in which enzymes involved in biosynthesis of fatty acids and/or desaturases are activated to fill disrupted membrane bilayers as a wound healing process (Myung *et al*, 2006). This activates LOX and this enzyme catalyze the first reaction of the complex metabolic pathway that leads to the formation of C6 and C9 volatile compounds (Feussner and Wasternack, 2002). The primary C6-green leaf volatile synthesized by the LOX pathway is (*Z*)-3-hexenal (Matsui *et al*., 2000), which is then converted to other

volatiles such as (*E*)-2-hexenal (leaf aldehyde), (*Z*)-3-hexenol (leaf alcohol) and (*Z*)-3-hexenyl acetate (leaf ester) (Shiojiri *et al*, 2006).

The autoxidation pathway is a non-enzymatic process that oxidizes unsaturated fatty acids like oleic, linoleic and linolenic acids to yield a mixture of hydroperoxides. This reaction is activated by free radicals with oxygen and it consists of three stages. An initiation, propagation and a termination stage. The reaction is initiated by initiators like metal ions and protein radicals which cause unsaturated fatty acids to form carbon-centered alkyl radicals (Jacobsen and Let, 2006). A free radical mechanism is propagated by the radicals in the presence of oxygen to form peroxyl radicals. The peroxy radical removes hydrogen from other lipid molecules and reacts with the hydrogen to form hydroperoxide and another lipid alkyl radical, dependent on the availability of oxygen and temperature (Velasco *et al*., 2003). If radicals react with each other, non-radical species are propagated and the reaction terminates (Choe and Min, 2006). The primary autoxidation product is hydroperoxide, however, further decomposition of hydroperoxide may occur in the presence of metals or oxygen leading to the formation of volatile secondary oxidation products including aldehydes, ketones, alcohols, hydrocarbons and furan responsible for off-flavor. For example, 2,4-heptadienal produced from linolenic acid has been reported to contribute to off-odor in fresh-cut iceberg lettuce (Palermo, 2012). Hence processing and post processing activities that expose wounded surfaces of produce to metals or oxygen may produce off-flavours early during shelf-life from autoxidation process.

Increased metabolic activity after processing leads to increased amino acids derived volatiles like 3-methylbutanal and 3-methylbutanol from leucine, however during storage their concentration decreases, as glycolysis and tricarboxylic acid cycle pathway become almost inactive alongside senescence (Aubert *et al*., 2005; Beaulieu, 2006). Glucosinolates, synthesized from certain amino acids are also natural precursors of flavour volatiles. Glucosinolates are sulfur-rich, nitrogen containing thioglycosides, commonly found in cruciferous vegetables like cabbage,

horse radish, rocket leaves, water cress, collards, mustard and kales. Endogenous thioglucosidases hydrolyzed after cutting, produces isothiocyanates and thiocyanates at neutral or high pH, and nitriles, at low pH (Bones and Rossiter, 2006; Chen and Andreasson, 2001; Husain, 2010). They have characteristic green, herbal, nutty almond-like smell (Jirovetz *et al*. 2002).

Phenolic volatiles may also be derived from the amino acid phenylalanine. These include 2, 4-bis-1,1-dimethylethylphenol (green smell) which can be found in lettuce and cabbage. The 2,4-bis-1, 1-dimethylethylphenol compound is produced by the phenylpropanoid acid pathway associated with browning when the product is sliced or cut (Lonchamp *et al*., 2009). However oxidative activities of the polyphenol oxidase (PPO), an enzyme responsible for browning, could induce a decrease in the concentration of this volatile due to its activity in senescence and off-odor production (Bassil *et al*., 2005; Nogueira *et al*., 2005).

Further research into volatiles produced within leafy vegetable tissues and their effects on senescence during minimal processing and post processing may be important to enhance product quality. In addition the metabolic processes for volatile production and the minimal processing step at which they are activated in various leafy vegetables requires further studies.

2.3.3 Changes in microbial quality

Microbial quality is another important factor in minimal processing, as products contaminated with micro-organisms are not visible to both processors and consumers unless detected. The major groups of microorganisms that can contaminate fresh and minimal processed products are bacteria, yeasts and moulds, but in some cases viruses like Hepatitis, or parasites like Giardia can cause major health concerns (Francis *et al*., 2003; Cantwell and Suslow, 1999). Mesophilic bacteria are the major microbial groups that contaminate horticultural products from the field, however low temperature conditions during minimal processing and storage stimulates a shift to psychrophilic bacteria, though both populations can be found in final products during microbial analysis (Pothakos *et al*., 2012). Initial raw

material microbial contamination is critical for the final minimal processed product quality. This is because minimal processing washing and disinfection activities can only reduce microbial contamination but not eradicate it. Reductions ranging from 0.7 – 2.7 log CFU/g have been reported using some treatments like cold water, chlorine, ozonated water, peroxyacetic acid and chlorine + ultrasound in increasing order of effectiveness (Baur *et al*., 2004; Allende *et al*, 2008; Seymour *et al*., 2002).

Secondly, the processing activity itself can contaminate the product. Minimal processing activities as trimming, coring, cutting, shredding and washing induce damages to the produce tissues and removes the protective layer of the epidermis of the produce (Martn-Belloso *et al*., 2006), exposing product surfaces to contamination by bacteria and other human handling pathogens. Cross contamination may also occur between product batches (Gil *et al*., 2009). The cut or wounded tissues exudes fluids and nutrients that also nourishes the microflora and enhance its proliferation (Heard, 2002). Moreover, the pH of most vegetables is close to neutral or even higher and favours bacteria growth. Yeast on the other hand thrive well in acidic environment (at low pH). As a result the growth rate of yeast on fresh cut vegetables is slow compared to bacteria (Gómez-López *et al*., 2008b; Jacxens *et al*., 2002). However while bacteria contamination above 10^8 CFU/g is needed to cause spoilage, lesser contamination level in yeasts and moulds (above 10^5CFU/g) can cause spoilage and produce off-odors which can be detected by consumers (Jacxsens *et al*., 1999; Ragaert *et al*., 2006).

2.4 Influence of minimal processing activities/ equipment on quality changes

The equipment required for minimal processing of fresh produce perform different functions during the various processing steps like cutting, coring, shredding, washing, and dewatering, influencing the final quality of the product. The operation at each step alters the integrity of the raw material especially in the cut products, making them more prone to deterioration (Sanz *et al*., 2002). Also, these different unit operations provide opportunities for cross-contamination, as a small lot of contaminated product may affect a large lot during the processing steps (Gil *et al*.,

2015). In addition due to leaching of nutrients and exudates, it is important to process different leafy vegetables in different processing lines or to carefully clean the lines before changing product. A classic example is cabbage which releases a high concentration of organic nutrients into washing water during processing (Cantwell and Suslow, 1999).

The main risk factors for product quality and safety are the temperature during processing, water quality and sanitation, hygienic design and hygiene status of equipments, as well as employee hygiene and training (Castro-Ibáñez *et al*., 2016). The main minimal processing steps and the effects of the various equipments used for fresh-cut processing are discussed in detail in the subsequent section. Figure 2.2 depicts the mostly practiced minimal processing steps and product handling operations.

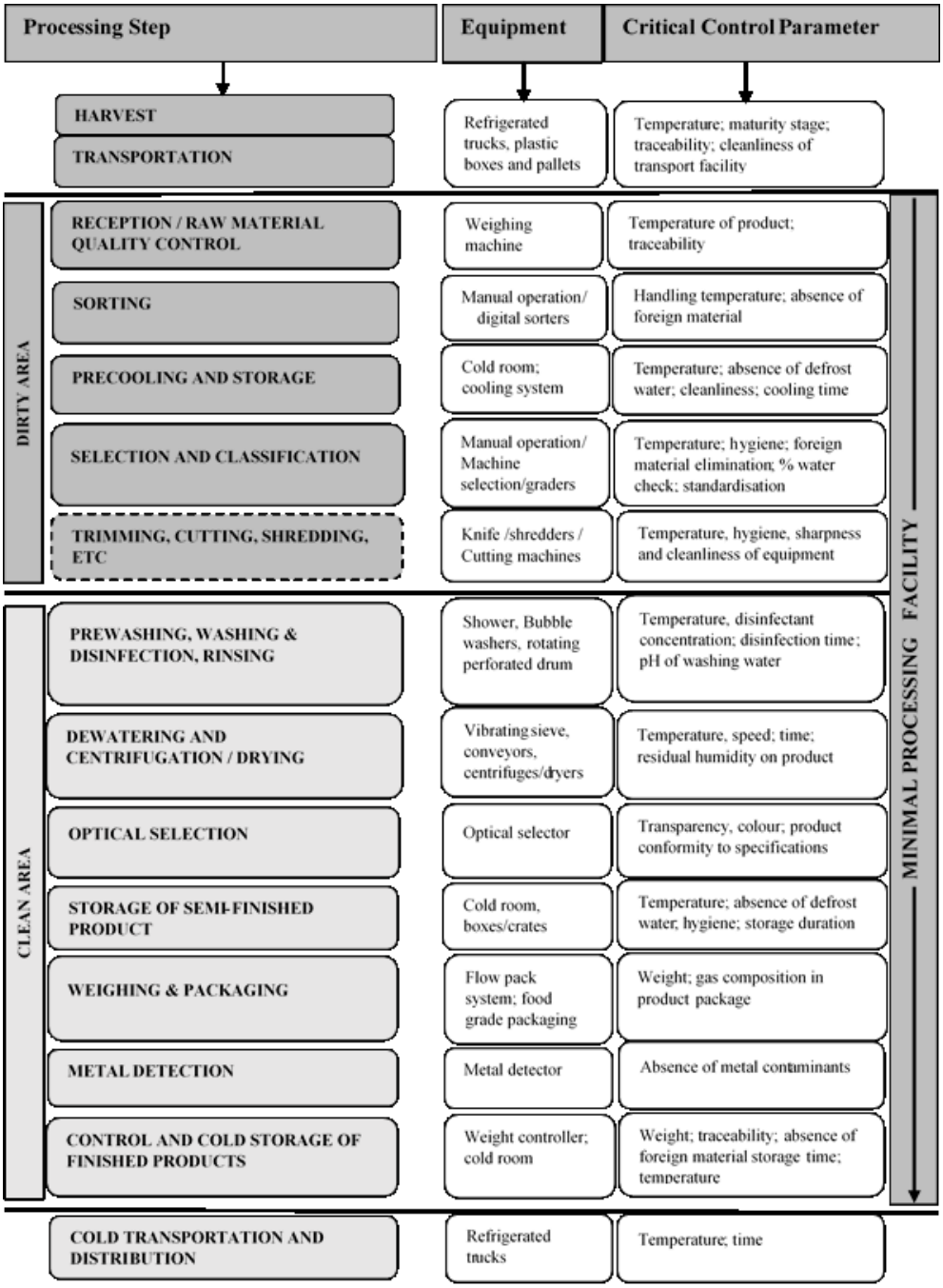


Figure 2.2: Minimal processing steps of leafy vegetables, equipment and quality control parameters (NB: trimming, cutting and shredding is absent during whole leafy vegetable processing)-Adapted from Project QUAFETY outcomes and Artes-Hernandez *et al*., 2014)

2.4.1 Cutting equipment

Cutting or size reduction activity could limit the stability and quality of fresh-cut products. The process breaks the surface epidermal layer of produce causing an increase in respiration, a release of phytonutrients while exposing the product surface to microbial contamination. The limitations related to this processing step includes, desiccation, microbial spoilage, browning of tissues, discoloration, development of off flavour and taste (Bansal *et al*., 2015).

Dicers, slicers, choppers, shredders and manual cutting operations exert different forms of stresses and injuries on cut products. Several studies have reported the effects of cutting on fresh produce (Izumi, *et al*., 1996; Barry-Ryan and O'Beirne, 1999; Ahvenainen, 2000; Artes, 2000; Saltveit, 2003; Aguayo *et al*., 2004; Simoes *et al*., 2009; Matthew, 2013). The choice and type of cut depends on the product's intended use in relation to the commercial standards, but a product may have severe or mild wound effects depending on the intensity of the cutting activity. Particularly the number of cuts, the sharpness of the blade and the severity of the cutting treatments may influence the final quality of the product (Artes-Hernandez *et al*., 2013).

As reported, shredded radish lost more soluble solids than sliced ones in cold storage (Saavedra del Aguila *et al*., 2006). Similarly, diced purple onions resulted in lower soluble solids, lower pH and higher acidity after storage at 0^{o} C than slices (Berno *et al*. 2014). In the same way, studies on the effect of 4 different types of cutting, showed that sweet pumpkins samples cut into 8 pieces among sliced, shredded and diced ones had the highest sensory score, lowest ethylene and CO_2 production (Lee *et al*., 2008). Lemons cut into wedges lost significant amounts of phenolic compounds compared to slices, 1/2 slices and 1/4 slices samples after shelf-life (Artés-Hernández *et al*., 2007), indicating the effect of number of cuts on taste and flavor and loss of phytochemicals. Notably, this step is a critical point that requires processing line hygiene. The equipment used for cutting need to be cleaned, disinfected, and sharpened at regular intervals every working day to avoid

the build-up of organic residues and microbial contaminant, as well as to reduce damage caused to the product (CAC, 2003; FDA/CFSAN, 2008). Barry-Ryan and O'Beirne (1998) showed the effect of blade sharpness on the severity of physical damage, physiological stress and microbial growth of a commodity as razor blade < sharp machine blade < blunt machine blade (razor blade cause the least damage). In confirmation, though the level of sharpness was not quantified, Grout *et al*. (2002) reported that maintaining cutting knives at a high level of sharpness, delayed the onset of enzymic browning on sliced green beans by up to one day in cold storage. Fresh-cut carrots prepared with sharp cutting blades also showed reduced wound response, lignin accumulation, white blush, softening, and microbial growth (Barry-Ryan and O'Beirne, 1998). Furthermore, scanning electron and fluorescence microscopic imaging showed that sharp blade cutting (thickness, 0.04 mm) of egg plants caused less physical injury and cell death, which reduced leaching of phenolics and its ensuing contact with polyphenol oxidase activity, hence reducing browning (Mishra *et al*., 2012). On the other hand, melon pieces cut with a blunt blade exhibited increased ethanol concentrations, off-odour, and electrolyte leakage compared to pieces processed with a sharp blade (Portela and Cantwell , 2001) The extent ofloss of ascorbic acid in iceberg lettuce have also been attributed to the cutting method and sharpness of the blade (Barry-Ryan and O'Beirne, 1999).

Besides sharpness, the type of blade itself and equipment used, may also influence cutting quality. Fresh-cut lettuce processed with sharp rotating blades was reported to have lower respiration rates and microbial counts during storage than those from sharp stationary blades (O'Beirne, 1995). Also, the use of new knife blades caused less damage compared to used and sharpened blades which caused red discoloration and whitening dehydration on cut romaine lettuce, after 12 days in air at 2.5 ^{o}C (Cantwell *et al*., 2016). The authors also recommended food grade water-jet cutting to have superior cutting quality (in terms of product visual quality and discoloration) than blade cutting when modified atmosphere packaging is not used.

Despite the effects of the cutting equipment, the severity of the cutting may also be influenced by the direction and may vary from product to product. However, research work with regards to cutting direction is not very extensive. Abe *et al*. (1998) reported that longitudinal cut direction produced banana slices that brown and soften rapidly and with higher respiration rate than those of the transverse cut direction. On the contrary, Deza-Derund and Petersen (2011) assessing the impact of cutting direction on respiration rate and volatiles formation reported that transverse cutting of lettuce through the mid-rib was a more severe method of preparation, which emitted volatiles of the lipoxygenase (LOX) pathway, while longitudinal cutting enhanced formation of volatiles from other metabolic routes.
Generally, selecting the right type of blade, using sharp blades and minimizing the severity of cutting would improve fresh-cut product quality, provided temperatures are low enough to minimize respiration and metabolism.

2.4.2 Washing equipments

Washing is done to remove foreign materials from whole of cut product surfaces. The washing and cooling of products directly after cutting reduces respiration, and minimize the injury responses by removing sugars, stress related compounds like acetaldehyde, phenols and other nutrients at the cut surfaces that may also favor microbial growth and tissue browning or discoloration (Cantwell and Suslow, 1999; Toivonen and Stan, 2004). Also the unknown signal elicited by wounding which initiates increase in respiration and its consequence effect on tissue softening, loss of flavour and browning is removed by washing (Cisneros *et al*., 2014). In addition, the cold water used during washing aids in preventing internalization and infiltration of bacteria (Sapers, 2003), however, it also increases cost of operations and may be a health hazard for employees (Maffei *et al*., 2016).

Washing aids in removing soil, dust and any agrochemical residues weakly bound to the surface of the leaves (Lopez-Fernandez *et al*., 2013). Nonetheless, loss of pesticide residue on the surface of leafy vegetables, is dependent on the solubility of the pesticide in water as described for diethofencarb on crown daisy leaves during

washing with stagnant and then running water (Kim *et al*., 2016). Chlorinated water used for disinfection, has also been found to be effective in removing pesticide residue on the surface of fruits and vegetables (Bajwa and Sandhu, 2014).

Among washing sanitizers, chlorine is the most widely used. It is relatively easy to use, costs low and is able to prevent pathogen cross-contamination of produce during washing (Lopez-Galvez *et al*., 2009; Luo *et al*., 2011). However, the potential generation of trihalomethanes (THMs), when chlorine or chlorine based sanitizers are used, may present health hazards. Fortunately, recent studies have reported that total THM levels in the vegetable tissue were below the detection limit (Gomez-Lopez *et al*., 2013). Moreover, chlorine-based sanitizers, used under optimal conditions, should not represent a high risk of THM formation (Artes-Hernandez *et al*., 2013). Most of the research studies on the use of sanitizer during washing have focused on microbial quality and reduction, with very little information of the effect on phytonutrients (Beltran *et al*., 2005; Martinez-Sanchez *et al*., 2006). Vandekinderen *et al*. (2007) report the effects of peroxyacetic acid of different concentrations on phytonutrients of cabbage. The use of low concentration of Chriox 5, a sanitising agent corresponding to peroxyacetic acid, in concentrations of 40 to 250 mg L-1 led to loss of total vitamin C content varying from 15 to 25%. However, loss of vitamin C could also be attributable to its hydrophilic properties. This is because decontamination effects with potable water or sodium hypochlorite (20 and 200 mg L-1) did not decrease the alpha- and beta-carotene content of fresh-cut carrots (Vandekinderen *et al*., 2007).

Maximum respiration rate of whole salad rocket and wild leaves has been reported after washing with different treatments cold water, ozonated water, ozonated water + UV-C and hot water; in increasing order of respiration (Martínez-Sánchez *et al*., 2008b); probably due to tissue shock during the washing activity. Oxidative action of disinfectant (chlorine or peroxyacetic acid), coupled with bubble action of washer may also cause browning or loss of green colour on the whole un-cut surface of leaves during storage. This could be related to Type II chlorophyll

breakdown caused by oxygen radicals related to fatty acid oxidation (Toivonen and Brummell, 2008). Optimizing washing operations could reduce these effects.

Usually washing systems in the fresh-cut industry are made up of three washing phases and tanks. However, depending on product and operating conditions of a company, the washing phases in tanks could also be single or double with various wash and spray combinations (Luo, 2007). The washing systems are sometimes termed as 'jacuzzi' due to the bubbling action they produce. The first wash removes all dirt, soil and debris combining both the sprayer and the washing tank activity in most cases. Water in this tank increases rapidly in microbiological load, requiring an implementation of a filtration and refreshing water system that respects the product-to-water ratio, and application of a disinfecting agent to keep the microbial load of the water at a low level (Lopez-Galvez *et al*., 2010; Holvoet *et al*., 2011). In the following tank a second wash is then applied. At this phase, any microbiological load on the fresh product is further decreased, however cross-contamination within a lot or among lots may occur (Luo *et al*., 2011). In this same tank, sanitation of the product takes place and the water is treated with an agent mainly chlorine to reduce microbial load and prevent cross-contamination during washing (Soliva-Fortuny and Martin-Belloso, 2003; FAO, 2008).

The turbulence or force of flowing wash water on salad surface mainly promotes the mechanical removal of microorganisms; however it may also cause slight structural damage of soft leafy vegetables. Besides, in cut products the surface may absorb wash water, making disinfection very critical to prevent contamination (Cantwell and Suslow, 1999). Despite the quantity of water used, the quality of water used in washing whole products impacts on the effectiveness of washing (Allende *et al*., 2008; Lopez-Galvez *et al*., 2009). The third and last washing phase before packaging is the rinsing step, which requires very low or no dose of disinfecting agent to achieve good results. Other commercial operations also adapt open flume and closed-flume systems (Luo, 2007). Recently, a patented system which has adapted the closed pipe flume concept, have been introduced to wash fragile and delicate products, such that contact time with sanitizing water solution

is precisely controlled for full immersion and appropriate treatment time (Turatti, 2015). This has been recommended, as it does not remove the bloom of blueberries and may be applicable for delicate baby leaf vegetables, as maintaining intact surface layer and the avoiding leakage of nutrients during the washing step seem to be a limitation.

Other washing systems including ozone washers which operates in two way, either by rotational movement to stir washing water or by mid-range ultrasonic waves to produce bubbling and effect cleaning in minimally processed vegetables have been proposed (Kim *et al*., 1999; Long *et al*., 2011)

2.4.3 Conveyors belts

Conveyor belts are one of the hotspots for microbial contamination (Buchholz *et al*., 2012). They help to transport produce from one equipment to the next one while contributing to complementary operation as foreign body elimination and dewatering. The types of conveyors which are mostly used in the minimal processing of leafy vegetables, are the belt conveyors and the vibratory conveyors. The belt conveyors are used for loading incoming products, serve as trim tables with flexible speed and are also used to transport fresh-cut products from the washer to the dryer. The vibratory conveyor of the horizontal motion type are characteristically cleaner than the conveyor belt due to its stainless steel make-up. However, vibratory conveyors are not suitable for leafy vegetables due to possible rubbing and damage on the shaker bed (www.key.net), and so are more suitable for products with friction. Conveyor belts are used for dewatering or surface drying of leafy vegetables that are too delicate for centrifugation/spin drying. Surface drying on the conveyor belt is achieved through passing forced chilled air circulating over a perforated belt that transports the products as in the use of air-bed conveyors which are widespread in use across Europe and the United States, although their efficiency to dry high volumes should be optimized (Artes and Artes-Hernandez, 2003; Turatti, 2011).

2.4.4 Drying Equipments

After washing, removal of gained moisture on the produce surfaces is done using several systems, which include draining devices, gentle removal with cheesecloth, centrifugal spin driers, vibrating racks, rotating conveyors, hydro sieves, forced air and spin less drying tunnels (Gorny *et al*., 2002). It is important that the drying process not only removes moisture gained during processing but also removes liquids from cell leakages that can support microbial growth and enzyme activity (Artés-Hernández *et al*., 2013). For leafy vegetables like lettuce, removal of slightly more moisture (i.e., slight desiccation of the product) may favour longer post-processing life (Cantwell and Suslow, 1999). This may also be true for rocket leaves as controlling the development of off-odors in packaged washed leaves during storage was related to the critical need for the complete removal of free water from leaves during the drying step (Rux *et al*., 2017).

Centrifugation or spin drying is widely used in the fresh-cut industry, although other methods such as vibration screen and forced air tunnel have also been adopted for water removal (Bolin and Huxsoll, 1990; Moretti *et al*, 2007). The high centrifugal force not only removes water, but it also cracks and crushes the tissues hastening senescence (Ahvenainen, 2000; Saltveit, 2003; Moretti *et al*., 2007). Water loss through damaged cells from the spinning process may also affect sensorial attributes like visual quality, taste and texture. It is therefore important to optimize speed and time requirements suitable for specific products to reduce quality losses during the process.

Several studies have been published on the effect of conventional drying systems on the nutritional quality of vegetable products. However, there are very few studies on the effect drying operations on the quality of phytonutrients in fresh-cut products. Although it has been reported that the retention of nutritional properties of leafy greens are better at a faster drying rate (Negi and Roy, 2001), the extent to which drying dynamics affect the product quality is unknown. In air-tunnel drying systems, heated dry air absorb moisture from the product which then pass through

a cooling unit which blows cold air before it exits the dryer. Though heated air is applied for a short period, high temperatures may induce several irreversible biological or chemical reactions which may cause modifications in color, decrease of sensory quality, loss of nutrients, aroma and texture (Abid *et al*., 1990). Despite this, if drying is done under controlled conditions with cold air, then the fresh properties of the product can be maintained (Nagaya *et al*., 2006); the only limitation is that, the air-dryers have low efficiency to dry high volumes of product (Artés-Hernández *et al*., 2013).

The use of predictive models like the multiphase transport model can be adapted to aid in improving drying efficiency as it is capable of predicting actual drying rates, operating conditions and it assures the absence of critical wet areas on product surfaces for microbial spoilage (Curcio *et al*., 2016). Other drying techniques that may be adapted include the use of low humidity air dryers, infrared air dryers (where infrared is used as the heat source) and radio frequency dryers (Naidu *et al*., 2016), taking note of their efficiency and ability to minimize chemical degradations and nutrient loss (Van Loey *et al*., 2005).

2.4.5 Packaging

This is the final step of minimal processing. During this step products are either weighed and packaged directly or temporary stored (0-12hours) in a cold room prior to packaging. Active modified atmosphere or passive atmosphere packaging are normally used in fresh-cut industry depending on the product needs. Active MAP is aimed to rapidly substitute air with the desired gas composition by gas-flushing or gas-scavenging or emitting systems added to produce N2, CO2, and/or to remove gases (e.g., O2, CO2) to speed up the achievement of the equilibrium. Passive MAP on the other hand is developed by the interaction of packaging film gas permeability and respiration of the product (Gavara *et al*., 2009; Zagory, 1999; Talasila and Cameron, 1997; Artes *et al*., 2006) and is used for product for which the use of gas composition different from air is less critical to the final quality and shelf-life, for instance, whole adult leaves where browning is not a limiting factor.

Modified atmosphere packaging (MAP) using a vertical or horizontal flow pack system is the common technique used. The packaging machines are usually made of round vertical tubes wrapped with tubular packaging material; the machine first seals the bottom part of the bag, fills it with the product, and then transports it in the internal part of the tube, by using weight-based portion control machines (Gil *et al*., 2015) and after filling it seals the upper part of the bags. These equipments may also have a gas mixer or filler such that minimal processed product exit with the appropriate gas compositions and packet weights. The primary gases used in modified atmosphere packaging are O_2, CO_2 and N_2. Although other non-conventional gases like argon, nitrous oxide and helium have emerged, they are still being tested and not introduced commercially.

Carbon dioxide is a colourless gas and has a slight pungent odor when it is used at very high concentrations. The high solubility of CO_2 can result in pack collapse causing the reduction of headspace volume. Generally, atmosphere with low O_2 and/or high CO_2 concentrations reduces the respiration rate, the growth of postharvest pathogens and deterioration rate during storage of fresh-cut products (Kader *et al*., 1989). CO_2 is the most important gas in the modified atmosphere packaging of foods, due its bacteriostatic and fungistatic properties. It inhibits the growth of the many spoilage bacteria and the inhibition rate is increased with increased CO_2 concentrations in the given atmospheres. CO_2 is highly soluble in water and fat, and the solubility increases greatly with decreased temperature (Sivertsvik *et al.*, 2002). However CO_2 is regarded as a competitive inhibitor of polyphenol oxidase (PPO) enzyme involved in the tissues browning through the hydroxylation of monophenols to *o*-diphenols and subsequent oxidation to *o*-quinone; a reduction of PPO activity using CO_2 atmosphere compared with storage in air was observed in cold stored plant tissues (Lattanzio, 2003). High CO_2 modified atmosphere has also been reported to significantly inhibit phenolic accumulation in fresh-cut lettuce and carrots due to ability to inhibit the phenylalanine ammonia lyase activity (Matteos, 1993; Amanatidou *et al*., 2000).

Oxygen is a colourless, odourless gas that is highly reactive and susceptible to combustion. It can promote deterioration such as lipid oxidation, browning reactions and pigment oxidation. Moreover, using low concentrations of oxygen can reduce the growth of microorganisms (O'Beirne et al, 2015). Nitrogen is a non-reactive gas with no odor, taste and color. Nitrogen alone does not support the growth of aerobic microbes but does not prevent the growth of anaerobic bacteria. The objective of MAP technique is take advantage of the combine effects of these gases; to create an optimum O2 and CO2 atmosphere around the packaged produce such that their metabolism and deterioration is retarded (Toivonen and Brummell, 2008). Usually, low O2 and/or high CO2 gas concentrations, decrease the respiration rate of the product, reduce the growth of postharvest pathogens, preserve the visual appearance, maintain nutritional quality, slows browning process and the rate of deterioration during storage (Kader et al., 1989; Gorny, 2004).

The optimal atmosphere concentration for most popular cut-products have been identified (Gorny, 1997), and there are many studies on the effect of gas composition and on packaging optimisation for several species as apple (Soliva-Fortuny *et al*., 2005; Aguayo *et al*., 2010), pear (Gorny *et al*., 2002; Gomes *et al*., 2012), tomato (Gil *et al*., 2002; Aguayo *et al*., 2004), pineapple (Marrero and Kader, 2006; Finnegan *et al*., 2013), mushroom (Simón *et al*., 2005), potato (Beltrán *et al*., 2005; Angós *et al*., 2008), kiwifruit (Cornacchia *et al*., 2008), honeydew (Bai *et al*., 2003; Zhang *et al*., 2013), mango (Beaulieu and Lea, 2003; Sarzi de Souza *et al*., 2006), carrot (Izumi *et al*., 1996; Simões *et al*., 2011).

However gaining the optimum atmosphere gas composition for a given product is not always possible and it will mainly depends on packaging film and storage temperature (Sandhya, 2010), that ensures an equilibrium atmosphere is reached in the package. An equilibrium atmosphere is attained when film permeation rates for O2 and CO2 match the respiration rates of the packaged fresh produce inside a package (Jacxsens et al., 2001; Almenar et al., 2007). This is critical for the success of MAP storage since exposure of fresh produce to high CO2 levels may cause

physiological damages while exposure to too low O2 levels may induce anaerobic respiration and the development of off-flavors (Zagory and Kader, 1988; Exama et al., 1993; Pretel et al., 1998; Pesis, 2005). The design and selection of the appropriate polymeric films, together with suitable trays of punnets and sealing is crucial (Artes et al., 2006; Artes and Artes-Hernandez, 2003; Martınez-Sanchez et al., 2011). Low density polyethylene, polyvinyl chloride and polypropylene are the main films used for packaging fruits and vegetables (Lee et al., 1996; Kader and Saltveit, 2003). They contribute to the prevention of desiccation and flaccidity due to vapour barrier properties and reduce the rate of senescence and re-contamination by microorganism (Brecht et al., 2004). MAP packages are checked periodically for seal integrity in water filled pressurized chamber.

Once a processor individuates the packaging material and dimensions for a given product, a variation in the respiration rate of the raw material may lead to unexpected and undesirable gas composition at the equilibrium (Sivertsvik et al.; 2002). This may be the case of products having variable respiration with the season, or if different varieties are alternated along the year. Tudela *et al.* 2013 indicated that CO_2 concentration in the packaging of fresh-cut lettuces from 6 different cultivars varied between 6.5 and 14.5 kPa at the end of storage, founding noticeable off-odours for some of them. The same authors found that a faster accumulation of CO_2 in the headspace of cut-products from immature heads than in over-mature ones, and an extreme variability with the in different months during the winter–spring seasons. As another example respiration rates of rocket leaves as found to vary with the season and the number of cutting (first, second, etc) or maturity. A variation from 86 to 34 mg CO2 kg-1h-1 at 10 °C has been reported by Seefeldt et al. (2012) passing from spring to late summer, and some differences are also caused by the number of plant cuttings (Martinez-Sanchez *et al.,* 2008 b). Other authors reported much higher respiration rates, decreasing with increase in maturity (Koukounaras *et al.,* 2007). The same variability has been reported for different variety and time of harvest of broccoli florets (Seefeldt *et al.*, 2012). All these factors suggest that respiration rate is a very critical factor to be monitored before

packaging, particularly in the case of different sources of raw materials. In addition, in case of any temperature abuse during transport, distribution and display, will induce an increase of product metabolism dramatically affecting the gas composition within the packaging. Shorter period of temperature abuse can, in fact, be detrimental to the final product quality and shelf-life, enhancing degradative reaction and the growth of microorganisms, with the consequent development of off-odors, as shown for several fresh-cut products (Kou *et al.*, 2014; Amodio *et al*., 2015; Luca *et al*., 2016; Amodio et al., 2017). The temperature recommended for storing fresh-cut products packaged in modified atmosphere is between 0 ^{o}C and 5 °C, but these products are often kept at temperatures of 10 to 12 °C, during display (Oliveira *et al*., 2010). Such temperature conditions pose also the risk of water condensation within packages due to poor gas exchange between the film, the product and the surrounding environment (Artes *et al*., 2006).

2.5 Conclusion

Critical assessment of the physiological changes that initiate degradation at critical processing points will be vital in designing methods to improve processing activities. This is because membrane breakdown induced by the processing operations is inducing critical changes in visual quality, colour, texture, flavour and taste of minimal processed leafy vegetables. The extent of the damage may vary with different type of equipments and different operation modes (washing conditions, conveyor belt speed, drying temperature and time, centrifugation time and speed, etc) and further studies may be aimed to intensely study these aspects. Moreover while some processing steps as washing and cutting are well studied, less is known about drying consequences. Finally, the impact of processing on sensorial and microbial quality are well studied, however the case of different sanitizing treatments and processing steps on phytonutrient retention, needs more investigation.

PART TWO: EXPERIMENTAL

Rationale of the study

Leafy vegetables form the majority of minimal processed products used by restaurants, food bars, hotels or directly by consumers. They contribute to health and nutrition due to their content of phytonutrients with antioxidant properties. However, minimal processing of these products into cuts, chops, shreds and or washing and drying to make them ready for use, increases their respiration and metabolic processes due to tissue disintegration and wounds, which may lead to losses of phytonutrients, discoloration of damaged areas, entry and contamination by microorganisms. As a result sensorial quality, shelf-life and marketability of final products are limited. This is a major challenge to the success and sustainability of the industry, however this situation may be intense or minimal based on the equipments and operation modes of processors. Though there are a number of research on minimal processing and quality as have already been reviewed in this study, there are still some uncertainties concerning the effects of the use of equipments and the operating modes on the nutritional, bio-active compounds, organoleptic quality and consumer satisfaction which has not been assessed holistically to include the shelf life and storage quality.

The general objective of this study was to determine the impact of the processing steps on nutritional, sensorial and microbial quality of minimally processed whole rocket leaves and cut lettuce.

Specific Objectives:

i. To assess the effect of minimal processing steps, operation modes and storage by different processors on the quality of rocket leaves.
ii. To assess the effect of minimal processing steps and operation modes on the quality of cut Iceberg Lettuce and Romaine Lettuce
iii. To evaluate the effect of drying treatments and residual surface moisture below 2 % on quality and shelf-life of minimally processed rocket leaves.

1.0 Effect of Processing Steps and operating modes on Quality Attributes of minimally processed rocket leaves and fresh-cut lettuces

1.1 General Introduction

Ready to eat fresh vegetable products have gained recognition and acceptance due to their, health, convenience, and nutritional characteristics. Rocket leaves and fresh-cut iceberg lettuce are the most representative species on the market, among fresh salads and other ready to eat vegetables. Rocket leaves have been found to be a rich source of phytochemicals like vitamin C, carotenoids, and polyphenols that contributes to reducing oxidative stress (Bogani and Visioli, 2007; Martinez-Sanchez *et al.*, 2008; Ostan *et al.*, 2015) and are mostly consumed raw in salad or cooked.

Fresh-cut 'Iceberg' lettuce, on the other hand, are low in vitamins, flavour and nutrients, however, they are characterized by a high-water and fiber content and by their crispy texture, making it ideal for salad mixtures and side dishes (Ansah *et al.*, 2015). Minimal processing without any addition of food additives is aimed to maintain the natural eating and nutritional properties of these products, while adding convenience in use. Fresh-cut products are usually sold in modified atmosphere packaging as a salad mix with other vegetables, or as a sole ingredient. With the growing market demand, several processing companies have emerged, but the major limitation affecting this industry is the high sensitivity of the raw material to microbial contamination and rapid deterioration (Artes *et al.*(2007).

Steps in minimal processing of leafy vegetables include selection, trimming (coring), cutting, washing, rinsing, dewatering and drying, and modified atmosphere packaging. Some authors report as the most significant steps affecting the quality of minimally processed vegetables are washing, cutting, disinfection and packaging (Artes and Allende, 2014). These steps have been identified to influence contamination, spoilage and storage quality (Barry-Ryan and O'Beirne, 1998, 2000; Artes and Allende, 2005a). Equipments used during processing operations may also

influence the effect of the steps on quality properties of the final product, but at the moment, there are very few studies available in literature assessing this issue. Over the years, equipment has been developed in response to processing characteristics of various leafy vegetables, since different kinds of equipments with separate functions are required for each step.

Typically, product morphology and style of trimming and cutting influence the design of the equipments. In addition, washing and drying,- equipments may also vary, depending on the product resistance to the stress, coupled with the processors investment choice for the systems (Lamikanra, 2002). The level of automation adopted by individual processors of ready to use leafy vegetables may, for instance, range from manual to completely automated, also depending on the company size.

During minimal processing, wounding stress caused by water agitation in the washing tanks or by cutting, may increase phenolic content and improve antioxidant activity of ready to eat products. However, according to Reyes *et al.*, (2007), wounding stress depends, on the balance between phenolic synthesis and oxidation. Besides, while wounding stress from abiotic activities (like agitation), may produce injury signals to induce the production of secondary metabolites (Reyes and Cisneros-Zevallos, 2003), cutting stress may cause breakage of the produce membrane enhancing reactions between oxidative enzyme systems and phenolic compounds resulting in oxidation of phenolics and browning (Saltveit, 2000).

Cutting leads to alteration of the internal composition of the vegetables as well as other physiological changes resulting in nutrient release from the wounded area during processing, which favours microbial contamination and affects flavour quality (Toivenon 1997; Rageart *et al.* 2007). Secondly, cutting leads to increased respiration rates which in turn may affect taste, flavor and other sensorial properties of leafy vegetables. Cutting of iceberg, romaine and butter leaf lettuce especially at the mid-rib area has been reported to increase total soluble phenolic acid derivatives (Ferreres *et al.*, 1997; Tomas-Barberan *et al.*, 1997) but also provides substrates for

polyphenol oxidase activity and browning. Furthermore, Dudareva *et al.* (2004) in a review reported that major progress has been achieved in plant volatile research studies through molecular and biochemical techniques; but little or no attention has been given to their significance to enzymatic and non-enzymatic browning; membrane deterioration (Deza-Durand and Peterson, 2011) and texture quality (Beaulieu and Lea, 2003; Beaulieu, 2006) during minimal processing.

Volatile organic compounds involved in biochemical reactions during processing and post processing of leafy vegetables have the potential to be used as quality markers. Typically, the accumulation of hydroxycinnamyl alcohols during processing steps of fresh-cut green asparagus indicates possible lignification during its post processing or storage (An *et al.*, 2007). Another example is the possible release of internal senescence volatiles like ammonia, due to cutting and washing stress, when the activity of phenylalanine ammonia–lyase (PAL) initiates lignification or proteolysis resulting from the injury even at low temperatures (Joy, 1988; Toivonen, 1997).

Regarding the washing step, the turbulent activity from the bubbling of wash water on the surface of salad leaves has been found to reduce microflora by approximately 1 Log unit, but it can also lead to loss of turgidity, surface dehydration and discoloration of leaves (Allende *et al.*, 2008). The washing activity may also increase phenolic metabolism and antioxidant vitamins through increased respiration. Rux *et al.* (2017) found out that respiration rate of washed rocket leaves (65. mg/kg h) was higher than its unwashed counterpart at (52mg/kg h). However, the effect of washing on respiration and phytonutrients may differ depending on whether the product is cut or processed as whole. In cut leafy vegetables, Vandekinderen, *et al*. (2007) found out that rinsing of fresh-cut white cabbage with potable water for 5 min caused about 20% loss in the vitamin C content, while in whole leafy vegetables like rocket leaves washing did not cause leaching of the glucosinolates or the activation of its degradative enzyme myrosinase (Bell *et al.*, 2017). Nonetheless, the stability of these compounds could be dependent on the pH

of the washing water (6-7) (Higdon *et al.*, 2007). The phytochemicals of vegetables are also related to the vegetable colour and flavour (Alarcón-Flores *et al.*, 2013; Cartea, 2010), suggesting that maintaining phytochemicals during minimal processing will aid in maintaining visual quality and taste of final products.

Despite the amount of research in this area, obtaining microbiologically safe products with good visual quality and nutritional values is still a challenge for processors (Bett *et al.*, 2001; Rojas- Graü and Martin-Belloso, 2008). Most research studies done in minimal processing are related to microbial assessment, equipment development for specific processing steps and/or storage evaluation (Das *et al.*, 2011; Turatti, 2015; Ragaert *et al*., 2007), whereas a holistic approach for assessing the efficiency of these equipments during minimal processing activities and their impact on quality of the final product is still lacking.

1.2 Materials and operating modes for whole rocket leaves and cut lettuce experiments

1.2.1. Rocket Leaves

1.2.1.1 Experimental design and Sample collection

The experiment design included 4 samplings in collaboration with four processors of fresh-cut salads, two in Italy and two in United States of America. Rocket leaves of 3 lots were provided by the processors for the experiment. Each lot was traced from raw material, through the processing steps to packaging, these varied with respect to the companies as shown below in Figure 1.1. Samples of about 150g - 200g per replicate (taken at random from different points in the lot) were collected for analysis under sterile conditions starting from the raw material prior to minimal processing and after the individual processing steps. Though the major minimal processing procedures were the same for all the companies, there were some differences in their processing steps and the type of equipment used, particularly for the number of washing tanks, type of disinfectant treatment and drying equipment used (Figure 1.1; Tables 1.1 - 1.4). All samples were placed in sterile re-sealable bags and transported to the laboratories of Postharvest research, University of

Foggia and Food Safety Laboratory of New Mexico State University in ice-boxes. Any damaged samples resulting from transportation were discarded. Samples belonging to common steps, highlighted in yellow in Figure 1.1, and particularly raw material (S1), sanitized product after the last rinsing (S2) and dried product (S3) were used for an overall comparison of the effect of processing steps, throughout different companies. Samples for each individual step were used for the evaluation of the effect of the processing operation in each company.

For Processor A and B also packaged samples were collected and evaluated after storage at 5 ^{o}C.

ITALY		USA		
PROCESSOR A	**PROCESSOR B**	**PROCESSOR C**	**PROCESSOR D**	
Raw Material	Raw Material	Raw Material	Raw Material	S1
*	Pre - Wash	Pre - Wash	Pre - Wash	
1st Wash	1st Wash	1st Wash	1st Wash	
2nd Wash	2nd Wash	2nd Wash	2nd Wash	S2
Tunnel dry	Tunnel dry	Spin dry	Spin dry	S3
Packaged samples stored at 5 ° C	Packaged samples stored at 5 °C	*	*	

Figure 1.1: Outline of the minimal processing steps for rocket leaves by four (4) different companies. *As shown in the table signifies an absent step for a processor. S1= First sample (Raw Material), S2 = Second sample (Sanitised product) and S3 = Third Sample (Dried or finished product).

1.2.1.2 Description of operational processing flow for Rocket leaves

i. **Raw material:** Rocket leaves were pre-cooled and temporarily stored for 1-3 days prior to processing.

ii. **Pre-wash:** This step was done to remove soil and debris from leaf surfaces. Sprayers or a combination of sprayer and washing tanks were used during this step.

iii. **1st wash:** Washing was done with a either chlorine-based or peracetic acid-based sanitizer. This is the main washing step which is supposed to remove microorganisms from the product by action of the water, whiles the sanitizing agent removes them in suspension and prevents cross-contamination of the product (Gil *et al*., 2009).

iv. **2nd wash:** At this step washing is repeated as in the 1st wash, however in this case, clean water is used. Some processors refer to this step as the rinsing step, because in most cases, the washing activity at this step is supposed to remove the disinfectant used during the previous washing activity. Water from this step is recycled back for use in the first washing activity.

v. **Drying:** Drying is aimed to remove the water added during the washing to the leaf surfaces. Drying was achieved using spin drier or air tunnels. In some companies, a dewatering step, preceded the drying. In this case, product was transported on a vibrating belt, to the drying tunnel. This step is critical to prevent microbial proliferation due to excess water when the product is packaged.

vi**. Packaging:** The minimally processed samples were packaged from all 3 sample lots of Processor A and B and collected for storage at 5^{o}C to assess the effect of distribution temperatures on quality. All samples were placed in sterile re-sealable bags and transported to the laboratories of Postharvest research, University of Foggia and Food Safety Laboratory of New Mexico State University in ice-boxes. Any damaged samples resulting from transportation were discarded.

Detailed description for each processor is reported in Tables 1.1 to 1.4. Processor A had two washing cycles, which were mainly done in two washing tanks for the sanitization step and the rinsing step respectively, without a pre-washing step; Processors B, C and D had three washing cycles which were a pre-washing step, a sanitization step and a rinsing step. Processor B used a chlorine based sanitizer while Processors A, C and D used commercial peroxyacetic acid-based sanitizers. Processors A and B used tunnels coupled with dewatering belts, while Processors C and D used the spin dryer for drying moist leaf surfaces (Figure 1.1). The temperature of the processing facilities during sampling activities ranged from 10-15^{o}C.

Table 1.1: Processing steps of Processor A

Processing Step	Product characteristics and processing conditions
Raw Material	Harvested, sorted and stored up to 24 hours prior to processing. The pre-cooling temperature of the produce was 3.0 ^{o}C. Measured produce temperature was 13.5 ^{o}C.
1st Washing	Temperature of washing water was 15 ^{o}C with a pH of 7.3. Sanitizing treatment was within the commercially allowed concentration for peroxyacetic acid (50 – 100 ppm)
2nd Washing	Temperature of washing water was 10 ^{o}C with a pH of 7.0. Clean water without sanitizer was used.
Drying	Dewatering temperature was 8.8 ^{o}C and product was tunnel dried at 30 ^{o}C. Drying time was 5 mins.
Packaging	Dried leaves were packaged immediately into polypropylene film bags to create a passive modified atmosphere.

Table 1.2: Processing steps of Processor B

Processing Step	Product characteristics and processing conditions
Raw Material	Harvested and sorted on the same day of processing. Measured produce temperature was 13 ^{o}C
Pre-washing	Temperature of washing water was approximately 16.4 – 17 ^{o}C for all the lots processed.
1st Washing	Temperature of washing water was 16.4 - 17 ^{o}C with a pH range of 6.9 -7.2. Sanitizing treatment was done with chlorine at< 0.1-ppm)
2nd Washing	Temperature and pH of washing water were the same as that of the previous washing step. Clean water without sanitizer was used.C
Drying	Product entered the tunnel dryer at 15 ^{o}C, drying is achieved at 35 ^{o}C, cooled to 7 ^{o}C and exited the dryer within 5-7 mins
Packaging	Dried leaves were packaged in plastic punnets and wrapped with polypropylene film to create passive modified atmosphere.

Table 1.3: Processing steps of Processor C

Processing Step	Product characteristics and processing conditions
Raw Material	Harvested, sorted and stored for about 3 days prior to processing. Produce temperature 4-5 ^{o}C
Pre-washing	Temperature of washing water was approximately 9-11 ^{o}C for all the lots processed.
1st Washing	Temperature of washing water was 9 - 11 ^{o}C. Sanitizing treatment was within the commercially allowed concentration for peroxyacetic acid (80 – 85 ppm)
2nd Washing	Temperature range and sanitizer concentration were the same as in the 1st Washing step.
Drying	Spinning time for the spin dryer used by Processor C was 3mins

Table 1.4: Processing steps of Processor D

Processing Step	Product characteristics and processing conditions
Raw Material	Harvested, sorted and stored for 3 days prior to processing. Room temperature was 4 -5 ^{o}C
Pre-washing	Temperature of washing water was approximately 9 - 11 ^{o}C for all the lots processed.
1st Washing	Temperature of washing water was 9 - 11 ^{o}C. Sanitizing treatment was within the commercially allowed concentration for peroxyacetic acid (30 – 80 ppm). Water temperature and concentration of sanitizer were tested every hour. During the processing there was a reduction in sanitizer concentration from 80 ppm to 40 ppm which was adjusted with 36 % O_2.
2nd Washing	Temperature range and sanitizer concentration were the same as that of the1st Washing step. However, reduction in concentration of this sanitizer from 80 to 50 ppm was adjusted with 28 % O_2.
Drying	Spinning time for the spin dryer used 3mins.

1.2.1.3 Quality parameters assessed on rocket leaves

All samples from the four (4) processors were analyzed for total phenol, total antioxidant activity, psychrophilic bacteria, yeast and mold and mesophilic bacteria count. However additional quality analysis were carried out, and this varied for each processor, according also to the laboratory availability. For Processors A and B, total vitamin C content (ascorbic and dehydroascorbic acid), titratable acidity, pH and total soluble solids were analyzed on the fresh samples, whereas aroma volatiles were analyzed on the frozen sample extracts. For processors C and D the samples were frozen in liquid nitrogen, then stored in -80 ^{o}C prior to the analysis, and were used for the determination of individual phenolic compounds (Sinapic acid and Ferulic acid). RT-PCR confirmatory test on total bacteria were also conducted only for samples from processors C and D.

1.2.2. Lettuces

1.2.2.1 Experimental design and Sample collection

The experiments were conducted in two cut leafy vegetables processing facilities, one in the Puglia region (Italy) providing Iceberg lettuce, and a second in Arizona (US), providing Romaine lettuce. Lettuce samples were traced from raw material through the processing steps to packaging which varied according to the company. Samples of about 200 g per replicate (taken at random from different points in the lot) were collected for analysis under sterile conditions for the raw material prior to minimal processing and after the individual processing steps reported in Table 1.5 and 1.6. Though the major minimal processing procedures were the same for both companies, there were some differences in processing steps and type of equipment used, particularly with regards to the number of washing steps and the type of drying equipment used (Tables 1.5 - 1.6). The temperature of the processing facilities during sampling activities ranged from 4 - 8 ^{o}C. In the case of processor A after drying, commercial samples packaged in modified atmosphere (2-3 % O_2 and 7 % CO_2) were also taken and stored at 5 ^{o}C to assess the effect of storage on quality. All samples were placed in sterile re-sealable bags and transported to the postharvest laboratory of University of Foggia or to Food Safety Laboratory of New Mexico State University in ice-boxes. Any damaged samples resulting from transportation was discarded.

1.2.2.2 Description of operational processing flow for lettuces

i. **Raw material**: Fresh harvested iceberg lettuce was stored one day while Romaine lettuce was stored up to 3 days before processing.

ii. **Size reduction:** The iceberg lettuce processed by Processor A was trimmed, cored, cut manually while the romaine lettuce processed by Processor B was cut mechanically.

iii. **Prewash:** This step was done to remove any contaminants on the cut surface of iceberg lettuce.

iv. **1st Wash:** This is usually done to decontaminate the product and reduce microbial load. Sanitizers were added to the washing water during this step. Recycled water was used during this washing step.

v. **2nd Wash:** Unrecycled water was used for the washing. Water from this point was recycled back for use in the first washing activity, after adding the sanitizing agent.

vi. **Drying:** Surface water from the washing activity was removed from the product at this point by using a 'humidity aspirator' this is a dewatering system, patented by the processing company, consisting in a perforated conveyor belt equipped with a pump alternating air blowing and aspiration to remove surface water without heat or a spin dryer. This was done to prevent microbial proliferation and to ensure that less wet product is packaged.

vii. **Packaging:** The minimally processed samples were packaged from all 3 sample lots of Processor A and collected for storage at 5°C to assess the effect of distribution temperatures on quality. All samples were placed in sterile re-sealable bags and transported to the laboratories of Postharvest research, University of Foggia. In the case of Processor B samples were not stored.

Detailed description for each processor is reported in Tables 1.5 and 1.6. For iceberg lettuce, size reduction was done by coring and cutting heads manually (Processor A). For romaine lettuce, the size reduction step was done mechanically and cut into slices (Processor B). Processor A, had three washing cycles, which were mainly a pre-washing step, a sanitization step and a rinsing step while, Processor B had two washing cycles which were a sanitization step and a rinsing step. Both Processors A and B used commercial peroxyacetic acid-based sanitizers. Another difference was at the drying step, Processor A used its in-company customized dewatering system defined as 'Humidity aspirator', while Processors B used a spin dryer.

Table 1.5: Processing conditions of Processor A (Iceberg lettuce)

Processing Steps	Product characteristics and processing conditions
Raw Material	Harvested, sorted and stored up to 2 days prior to processing. The pre-cooling temperature of the produce was 2.9 ^{o}C
Coring and cutting	Produce temperature was approximately 3.6 ^{o}C for all the lots processed.
1st Washing	Temperature of washing water was 14.2 ^{o}C with a pH of 7.3. Sanitizing treatment was within the commercially allowed concentration for peroxyacetic acid (50 – 100 ppm)
2nd Washing	Temperature of washing water was 12.1 ^{o}C with a pH of 7.4. Similar sanitizer concentration used in the 1st Wash was also used at this point.
Drying or Dewatering	Drying time for the 'humidity aspirator' (blower and suction process) used by Processor A was 50 seconds
Packaging	Dried samples were packaged directly into polypropylene film bags with active modified atmosphere

Table 1.6: Processing conditions of Processor B (Romaine lettuce)

Processing Step	Product characteristics and processing conditions
Raw Material	Harvested, sorted and stored up to 3 days prior to processing.
Coring and Slicing	Produce temperature was approximately 4.4 ^{o}C for all the lots processed.
1st Washing	Temperature of washing water was 3.8- 4.4 ^{o}C. Sanitizing treatment was within the commercially allowed concentration for peroxyacetic acid (30 – 80 ppm)
2nd Washing	Similar temperature range and as in the 1st Wash was used at this point, without a sanitizer.
Drying	Spinning time for the centrifuge or spin dryer used by Processor B was 3minutes.

1.2.2.3 Quality parameters assessed on lettuces

Microbial analysis, total phenolic and antioxidant activity were carried out for both Iceberg and Romaine Lettuces. In addition, for Iceberg lettuce, Vitamin C, pH, total soluble solids and volatile analysis were assessed, whereas, glucose content and phenolic composition and RT-PCR on Total Bacterial Screening were assessed for the Romaine lettuce.

1.3 Methods

1.3.1 pH, Titratable acidity, Total Soluble Solids (Brix) and Glucose content

Samples were homogenized at an average speed for 1 min (Ultraturrax T-18 IKA Basic; Wilmington, NC, USA) and filtered to extract juice. A 1 mL aliquot of juice was diluted with 40 mL of distilled water. The pH value and the titratable acidity were measured with an automatic titrator (T50 M Terminal, Mettler Toledo, Switzerland). Acidity was determined by titrating the juice with 0.1 N NaOH to an end point of pH 8.1 and expressed as percentage citric acid. Total soluble solids (in % Brix) was determined by measuring the refractive index using a digital hand refractometer (RX-7000cx; Atago Co. Ltd., Japan).

Glucose content: A glucose standard stock solution of 1.0 mg mL^{-1} was prepared with LC-MS water (Optima LC/MS) and stored under refrigerated conditions. Samples were juiced, centrifuged at 4000 rpm for 20 minutes and filtered through activated C18 cartridges. Samples were then filtered through 0.2 µm pore size membrane filters and then diluted 1:1 with LC-MS water. Before use, the LC/MS was tuned and calibrated with sodium iodide solution 2 µg/µL (NB: sodium iodide covers the required mass range, does not persist in the ESI source due to its volatility and does not interfere with the sample). The solution was infused using a syringe pump connected directly to the ion-source. HILIC/MS analysis was carried out on an ACQUITY HPLC system (Waters Corp., Milford, MA, US), coupled to an ion-trap mass spectrometer (Micromass Quattro Ultima, Mass Spec Detectives, Inc.) equipped with an electrospray interface. Chromatographic separation was performed on the ZIC-HILIC stationary phase column (150 x 2.1 mm, 3 µm

SeQuant, Darmstadt, Germany). The flow rate was 0.2 mL/min, the injection volume was 5 µL and the HILIC column was used at a temperature of 30ºC. The solution used for washing the syringe and injection volume was acetonitrile/water (95:5, v/v). Mobile phase A composed of 5mM ammonium acetate modified with formic acid to a pH of 4 and mobile phase B was 0.1 % formic acid in acetonitrile (v/v). The gradient elution profile started with a linear increase from 10 % B to 90 % B in 19 mins, according to the method of Antonio *et al*. (2008) with slight modifications. The mobile phase was allowed to return to the starting conditions in 1min, followed by column re-equilibration of 10 mins. The ion-trap mass spectrometer was operated in the negative mode with ion-source voltage set at -3.0 kV, capillary voltage of -20 V and temperature of 300 ºC. Mass spectra scan range was 100-1000 m/z. Nitrogen was used as drying, nebulizing, and collision gas. Glucose content (225 m/z) was determined at 13.1 min of retention time.

1.3.2 Phenol compounds, Antioxidant activity and Vitamin C

Five (5) g of each sample replicate step were homogenized at an average speed for 1min (Ultraturrax T-18 IKA Basic; Wilmington, NC, USA) in a buffer solution of 80 % methanol and 2 mM sodium fluoride. The homogenate was filtered and centrifuged at 10000 rpm for 5 min at 4 ºC. The resulting supernatant was used for the following analysis.

Total phenols: The method of Singleton *et al*. (1965) was used with minor modifications, by adding 100 µL of diluted supernatant to 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 gL^{-1}). Samples were then kept in a dark chamber for 2 hours before measuring absorbance at 725 nm against a blank (UV 1700, Shimadzu, Kyoto, Japan). Total phenols were calculated and expressed as milligrams Gallic acid equivalent per 100 g fw tissue.

Phenolic composition: The supernatants were collected and filtered through 0.2 µm pore size membrane filters for UPLC –ESI-QTOF-MS analysis. The liquid chromatography analysis was performed on ACQUITY UPLC system (Waters Corp., Milford, MA), including a degasser, an autosampler, coupled to qTOF mass spectrometer (Micromass Q-ToF Micro, Mass Spec Detectives) equipped with an electrospray ionization source. Chromatographic separation was carried out using an AccQ-Tag Ultra (2.1 x 100 mm, 1.7 µm particle size, 130 Å pore size, Waters, USA) column at 30 ^{o}C. The mobile phases consisted of 3 % acetic acid (A) and Methanol (B) (adapted from Zhang *et al*., 2013) at a flowrate of 0.4 mL/min. All solvents used were LC/MS grade. The gradient elution profile increased from 10 % B (12 mins) to 100 % B in 15 mins and after 17 mins returned to its initial conditions. The column was re-equilibrated for 5 mins. The injection volume was 5 µL. The pressure limit ranged from 0 to 15000 psi. Calibration curves of chlorogenic acid, p-Coumaric acid, ferulic acid and sinapic acid standards ranged from 0.0078 mg/mL to 0.25 mg/mL. MS analysis was carried out using electrospray ionization (ESI) interface in positive ionization mode. MS parameters were as follows: capillary voltage 3000 V, fragmentor voltage 160 V and drying gas temperature 300 °C. The instrument was operated in positive ion mode scanning from *m*/*z* 100 to *m*/*z* 1000 for detection of compounds. Nitrogen was used as drying, nebulizing, and collision gas. The mass data of the molecular ions were processed using Waters Mass lynx v.4.1 software. Ferulic acid (195 m/z) and Sinapic acid (225 m/z) were detected at 7.97 mins and 10.22 mins respectively for rocket leaves whiles Chlorogenic acid (355 m/z) and p-Coumaric acid (165 m/z) were detected at 2.34 mins and 5.16 mins for Romaine lettuce. All samples were analyzed in comparison with pure standards and expressed as mg per 100 g of fresh weight (mg $100g^{-1}$). ***Antioxidant activity***: The methods of Brand-Williams *et al*. (1995) using DPPH (α, α-Diphenyl-β-picrylhydrazyl) free radical scavenging method was used. The assay measures the scavenging capacity of antioxidants in the sample based on it reaction to DPPH. 50 µL of diluted extract was added to 950 µL of DPPH (0.04 gL^{-1} or 0.4 mM) daily solution. The activity was read after 24hours in the dark at

515 nm absorption, calculated and reported in milligram Trolox equivalent per 100 g fw tissue.

Vitamin C: Quantitative and qualitative analysis of ascorbic acid (AA) and dehydroascorbic acid (DHAA) was achieved as described by Zapata *et al*. (1992), with modifications. Five grams of rocket leaves were homogenized with 10 mL of MeOH/H2O (5:95) plus citric acid (21 g L^{-1}) with EDTA (0.5 g L^{-1}). The homogenate was filtered through cheesecloth and C18 Baker bond SPE column (Waters, Milford, MA, USA). The HPLC analysis was achieved after derivation of DHAA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine hydrochloride (OPDA). This was done by adding 250 µL freshly prepared OPDA solution to rocket leaves extract (750 µL) and allowing to react for 37 min prior to analysis. Samples of 20 µL were analyzed with an Agilent 1200 Series HPLC (Waldbronn, Germany). The HPLC system consisted of a G1312A binary pump, a G1329A auto sampler, a G1315B photodiode array detector from Agilent Technologies which detected DHAA and AA at 348 nm and 261 nm wavelengths respectively. Separations of DHAA and AA were achieved on a Zorbax Eclipse XDB-C18 column (150mm x 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. Pure standards were compared to rocket leaves extract for the determination of retention times and the quantification of ascorbic acid (AA) and dehydroascorbic acid (DHAA). AA and DHAA contents were expressed as mg ascorbic acid or dehydroascorbic acid per 100 g of fresh weight (mg $100g^{-1}$).

1.3.3 Aroma volatiles compounds and headspace SPME GC-MS analysis

Aroma volatile compounds were analyzed on frozen homogenized extract of each processing step replicate. The choice of making extract and freezing was also due to the time limitation in analyzing all the samples in the processing day. Thirty grams of rocket leaves were homogenized with 30 mL of distilled water for 6 minutes, after adding 0.6 g of $CaCl_2$ and 6 g of NaCl. For each replicate, 8 g of homogenized sample was weighed into a 20 mL solid phase micro extraction vial. Then a 2 µL of an internal standard solution (IS, 2-methyl-1-pentanol, 100 ppm) was added, and vials were immediately capped and frozen at -20 °C until analysis (max. 2 weeks in storage). Before the analysis, the sample vial was thawed 10 mins prior to heating at 40 °C in the GC-MS auto sampler for 20 mins. After heating time, an 85 µm fibre carboxen /polydimethylsiloxane (CAR/PDMS, Stable flex 24Ga, Manual Holder (Light Blue), SUPELCO, Bellefonte, PA, USA) was exposed for 30 min to the vial headspace and introduced into the GC injector port for desorption at 250 ^{o}C, for 4min in the split injection mode (1:20). An Agilent gas chromatograph model 7890A series coupled to an Agilent 5975C VL mass selective detector (Triple-axis detector) was used. Analytes were separated on a DB-WAX capillary column (60m x 250 µm internal diameter x 0.25 µm film thickness, Agilent technologies, Santa Clara, CA, USA) by applying the following temperature program: 40 ^{o}C for 4 min, heating up to 140 °C at 3 ^{o}C/min, with a final holding time of 10 min. Transfer line temperature was 280 ^{o}C. Mass detector conditions were: electronic impact mode at 70 eV; source temperature 230 ^{o}C; scanning rate 2.88 scan s^{-1}; mass scanning range m/z 30 – 400. The carrier gas was helium at 1.0 $mLmin^{-1}$. The identification of volatile compounds was achieved by comparing the mass spectra with the data system library (NIST 02, p>80). Data was processed using the Enhanced ChemStation MSD data analysis tool (GCMS 5975, Agilent technologies Inc.)

1.3.4 Headspace Gas Analysis for modified atmosphere (MAP) stored samples

The concentration of oxygen O_2 and carbon dioxide CO_2 gases in film bags was determined after packaging and after three days of storage at 5^{o}C using a gas analyzer (Dansensor, Model Checkmate 3, Denmark) which sampled the gas through a needle, using a built-in pump.

1.3.5 Microbiological analysis

Samples were collected directly into sterile bags to minimize external contamination. The packages, weighed area and weighing scales were sterilized (70% ethanol or IOSAN 25 ppm) prior to the analysis. Samples were then diluted 1:10 with saline buffer and homogenized for 2 min in a stomacher. Six decimal or serial dilutions (10^{-1} to 10^{-6}) were made and analyzed for aerobic mesophilic and psychrophilic counts, molds and yeasts. The bacteria micro-organisms were counted in Plate Count Agar (Fluka Analytical, Spain) kept at 35 ^{o}C for 48 hours (for mesophilic) and at 5-7 ^{o}C for 7-10 days (for psychrophilic). For yeast and molds, Potato Dextrose Agar (OXOID CD0139, Basingstoke, Hampshire, England) with 0.1 % chloramphenicol was used and kept at 25 ^{o}C for 5 days. Colonies were enumerated and counts were reported as log CFU/g by transforming microbial counts to logarithms.

RT-PCR analysis for Total Bacteria Screening; PCR templates were prepared using the spin-boil method. 1mL of 1:10 or 1:1 homogenate were transferred into a micro centrifuge tube and heated for 10 mins at 100 ^{o} C. The samples were then centrifuged at 12000xg for 1min, cooled in a cold block and the supernatants removed and saved as DNA template at -20 ^{o}C until the RT-PCR analysis. The reaction for DNA was carried out by preparing a master mix of 20 µL (10 µL of sterile PCR grade water, 5 µL 5xF1PCR Master mix and 5 µL TBS 5x oligonucleotide) for each DNA template replicate and adding 5 µL of thawed template in smart cycler tubes, on a cold block. The tubes containing the reaction mixture were spin down and then inserted into the smart cycler for analysis. Protocol for PCR was 150 seconds at 95 ^{o}C (denaturation), followed by 30 cycles

of 95 ^{o}C for 10 seconds and 58 ^{o}C for 30 seconds (amplificaton) and 40 ^{o}C for 20 seconds (cooling).

A fluorescent probe (**FAM Dye Set**) was used to monitor the amount of product at the end of each cycle and the real-time PCR instrument (SMARTCYCLER-***Processing Block, Cepheid, Sunnyvale, CA, USA)*** expressed the cycle in which it first detected fluorescence. The greater the number of starting copies (bacterial DNA template), the fewer cycles required to achieve fluorescence detection. Data analysis was done with the ***Smart Cycler System Software***. A fluorescent cycle threshold (Ct) unit of 15 setting was used for interpretation of results; Positive result, Ct ≥ 15; negative result, Ct < 15. Negative controls were included in each run.

1.4 Statistical data analysis

All data on concentrations of phytochemicals and glucose content were converted to dry basis (mg/g) calculated on raw material before washing, to avoid errors in reporting due to the additional moisture on the leaves.

Statistical analysis was done using general linear model (GLM) procedure with STATGRAPHICS Centurion software (XVI.I version, Stat point Technologies, Inc., 2009). A general linear model (two-way random effects model for processor and processing steps) was used to analyse the impact of the different processing steps on the quality of rocket leaves and the variability in quality of minimally processed leaves by the different processors. The box and whisker plot was used for graphical presentation of the distribution (median and inter-quartile range) of phytochemical content and microbiological quality of rocket leaves among processors and the variation/changes in the observed frequencies for the major minimal processing steps. Additionally, for each company, the effect of processing steps on quality properties of the rocket leaves and lettuces were tested by using One-way anova (fix effect model). Mean separation was achieved by applying Tukey's honest significance difference test ($p<0.05$). Hierarchical Cluster dendogram and heat maps were carried out after standardising the data, using the

ClustVis web tool for visualising multivariate data (Metsalu and Vilo, 2015) to determine the relationships between changes in phytochemical, volatile compound groups (only in the cases of Processor A and B – rocket leaves; Processor A – lettuces) and microbiological quality of minimal processed products and processing steps of the different processors. The Euclidean distance was used to determine similarities and dissimilarities between minimal processing steps and the quality variables.

Production and changes in volatile organic compounds were analysed using Principal Component Analysis (PCA). PCA was used to detect clustering and to investigate possible relationships between different processing steps and volatile compounds, using Statistica software (ver.7, Stat Soft, Tulsa, OK, USA)

1.5 Results and discussion

1.5.1. Rocket Leaves

1.5.1.1 Raw Material

Raw material quality for minimal processing is well known to influence the final quality of product and shelf life of leafy vegetables (Artés and Allende, 2005a; Francis *et al.*, 2012). Quality of rocket leaves prior to minimal processing varied among the four different processors from different locations (Table 1.7). Accumulation of phenolic in the raw material depends on several factors, including genetic variations, environmental factor, cultural or production practices and various forms of stresses (Parr *et al*., 2000). Phenolic compounds have been suggested to have antimicrobial activity (Cartea *et al*., 2010; Silva-Beltrán *et al*., 2015), and this can explain comparatively lower microbial counts (Table 1.7) for processors B and C, which showed higher total phenolic content (16.45 and 19.03mg gallic acid/g d.w., respectively) compared to processors A and D (5.99 and 12.20 mg gallic /g d.w.

Typically, mesophilic bacteria constitute the major group of microorganisms in leafy vegetable raw material, however their population usually correlates to psychrophilic bacteria during cold temperature storage as it favors their growth (Pothakos *et al*., 2012). As observed, though mesophilic bacteria count among the processors were significantly different, they corresponded to psychrophilic bacteria counts. Among the processors, microbial counts in raw material prior to processing did not exceed the maximum limit of 8 log CFU/g (mesophilic and psychrophilic bacteria) and or was lower of about 5 log CFU/g for yeast and moulds as expected in fresh processed vegetables (Debevere, 1996 and Jacxsens *et al.*, 1999), with count of processor A, slightly higher than in the other companies (Table 1.7). Yeasts and molds counts above 5 log CFU/g can cause spoilage and produce off-odors (Debevere, 1996 and Jacxsens *et al*., 1999).

Table 1.7: Quality characteristics of raw materials

Processor	Phenols mg gallic acid/g dry wgt	Antioxidants mg trolox/g dry wgt	Mesophilic bacteria log CFU/g	Psychrophilic bacteria log CFU/g	Yeast and Molds log CFU/g
A	5.99 ± 0.53c	5.12 ± 0.87b	5.37 ± 0.05b	6.75 ± 0.10°	5.17 ± 0.09a
B	16.45± 1.03a	14.99 ± 1.14a	4.86 ± 0.07c	3.51 ± 1.08b	4.87 ± 0.09b
C	19.03± 1.58a	9.09 ± 1.78ab	3.64 ± 0.01d	2.88 ± 0.41b	3.20 ± 0.12d
D	12.20± 0.35b	10.90 ± 4.08ab	5.63 ± 0.09a	5.62 ± 0.19°	4.27 ± 0.01c

Values are means ± standard deviation of triplicate samples (n=3). Values not followed by the same letters in columns are not significantly different, while values followed by different letters in a column are significantly different at ($P<0.05$)

1.5.1.2 Effect of minimal processing steps on quality attribute of rocket leaves

The results in Table 1.8 shows the effects of the processing steps, processors and their interaction on quality of minimal processed rocket leaves. The choice of using two-way random effects models was due to the difference in the processing steps and operation modes of each processor and the aim to estimate which factors (treatment) were affecting the variance among groups and not to quantify the magnitude of this effect on each group. Total phenolic and antioxidant content of rockets leaves (raw material, sanitized product and the dried product) were significantly ($p\leq0.05$) affected by the processing steps, processors and mostly by the interaction between these 2 factors ($p\leq0.0001$ and $p\leq0.001$). Though mesophilic

and psychrophilic bacteria counts were not significantly affected by the processing steps; the processors operations and its interaction with the processing steps resulted in significant effects (Table 1.8). Yeast and mold counts on rocket leaves were significantly affected both by processor and by the processing steps Table 1.8).

Table 1.8: Effect of major processing steps, processors operations and their interactions on the quality of minimal processed rocket leaves

Quality characteristics	Processing steps	Processor	Processing steps x Processor
Total Phenols mg gallic acid/g dry wgt	*	**	****
Total Antioxidants mg trolox/g dry wgt	*	*	***
Mesophilic /Aerobic Plate Count log CFU/g	ns	*	****
Psychrophilic bacteria log CFU/g	ns	**	***
Yeast and Molds log CFU/g	**	***	ns

Significance: ns, *, **, *** and **** = not significant, significant at $P \leq 0.05$, 0.01, 0.001 and 0.0001, respectively.

Although the raw materials were not the same and processing steps were different, some similarities were found among the 4 processors as shown by multivariate analysis of the data (Figure 1.2) The operations of Processors A and D had the most similar effects on total phenols, antioxidants and microbial quality of sanitized product and dried product.

Operations of all the processors resulted in total phenol content in rocket leaves increasing to the highest concentration in the sanitised product (Table 1.8, Figure 1.2 and 1.3). The median (50%) of total phenols was 24.77 mg Gallic acid/g d.w. in the sanitized product compared to median values of 14.34 mg Gallic acid/g d.w. in raw material and 15.31 mg Gallic acid/g d.w. in dried product (Figure 1.3). The increased total phenol content in the sanitized product could be related to effect of the turbulent washing procedure, which may have imposed some wounding stress on surface membrane of the rocket leaves. This is in accordance to the finding that increase in phenolic metabolism is related to wounding stress on produce surface. (Tomás-Barberán *et al.* 2000; Alarcón-Flores *et al.* 2014). Concerning the final dried products, similarities were observed in the total phenol content of products

from processors C and D, and processors A and B (Figure 1.2); though the content of total phenols were lower in the dried products compared to the sanitized products, they still remained higher than that of the raw material prior to processing (Figure 1.3).

Phenolic compounds have been found to correlate to antioxidant activity in rocket leaves and other plant species (Vallejo *et al.*, 2003; Moreno *et al*., 2006) and as such, the median of total antioxidant activity in sanitized products from all the processors, reached the highest level of 20.56 mg trolox eq. /g d.w compared to values of 9.99 mg trolox eq. /g d.w. and 12.01 mg trolox eq. /g d.w. of raw material and dried product, respectively (Figure 1.3). The operations of processors B and D had similar effect on the antioxidant activity of dried products. However, it should be noted that antioxidant activity of dried products in processor C contributed mostly to the high median of antioxidant activity in dried product (12.01 mg trolox eq. /g d.w.) compared to raw material (Figure 1.2 and Figure 1.3). The observed reduction in total phenol concentrations and antioxidant activity in the final dried product compared to the sanitized product, could probably be due to the effect of heat during the tunnel drying or to spin drying operations leading to chemical changes and mainly to oxidation that altered their respective contents (Klein and Kurilich, 2000).

Processing effects on microbial population were opposite to that observed for phytochemicals. Generally, the sanitization process that induced an increase of phytochemical content was also effective in reducing microbial load on the rocket leaves (Figure 1.2 and 1.3). Microbial counts on raw material prior to minimal processing were similarly reduced by the operations of Processor A, B and D, nonetheless initial microbial counts of raw material influenced the final load of the processed leaves. Conversely, in the case of samples from processor C, cross contamination may have occurred during the processing steps, most probably during 1st Wash and 2nd Wash and spin drying especially for psychrophilic bacteria which successfully thrive under low temperature conditions (Figure 1.2). Cross

contamination during minimal processing has also been reported by (Artés and Allende, 2005b). This also influenced the median values such that the level of psychrophilic bacteria on rocket leaves among the different processors were 4.57 log CFU/g on raw material, 4.12 log CFU/g on sanitized product but 4.76 log CFU/g on dried product. In the case of mesophilic bacteria, and yeast and mold counts, microbial load on final dried rocket leaf products began to increase compared to their corresponding sanitized product, though it was still below that of the raw material. Mesophilic bacteria showed a reduction from the raw material level of 5.11 log CFU/g to 4.24 log CFU/g in the sanitized product and 4.55 log CFU/g in the dried product with similarities also in the dried products of B and D processors (Figure 1.2).

Likewise, the median level of yeast and molds count on raw material of 4.57 log CFU/g among the four processors reduced to 3.87 log CFU/g and 3.91log CFU/g after sanitization and drying respectively. (Figure 1.2 and 1.3). The reduced level of microbial load after sanitization, which increased slightly in the dried product could be attributed to growth of returning epiphytic microorganisms (Turatti, 2015). Despite this, the final dried product did not exceed the maximum limit for mesophilic bacteria of 6 log CFU/g as established by the Spanish microbial regulation of fresh-cut vegetables on the day of consumption (RD 3484, 2000).

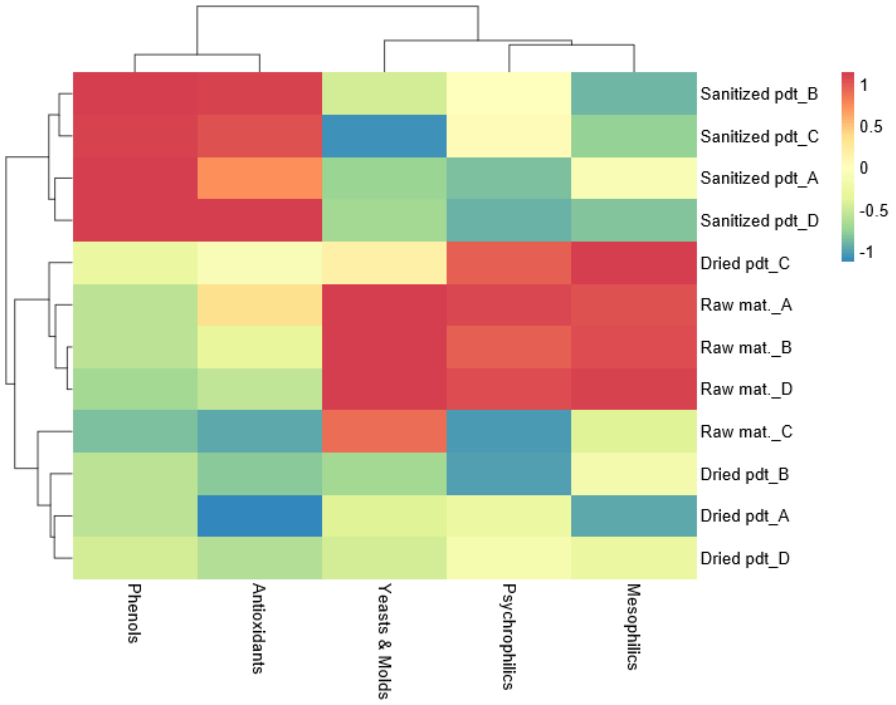


Figure 1.2: Cluster analysis and heat maps of phytochemical and microbial quality of rocket leaf products by processor A, B, C and D after the 3 main processing steps (Raw material-Raw mat, Sanitization (product after 2nd Wash) - Sanitized pdt and Drying – Dried pdt). The scale moves from red (high) to blue (low)

1.5.1.3 Dynamics of Rocket leaf quality characteristics resulting from each processor mode of operation

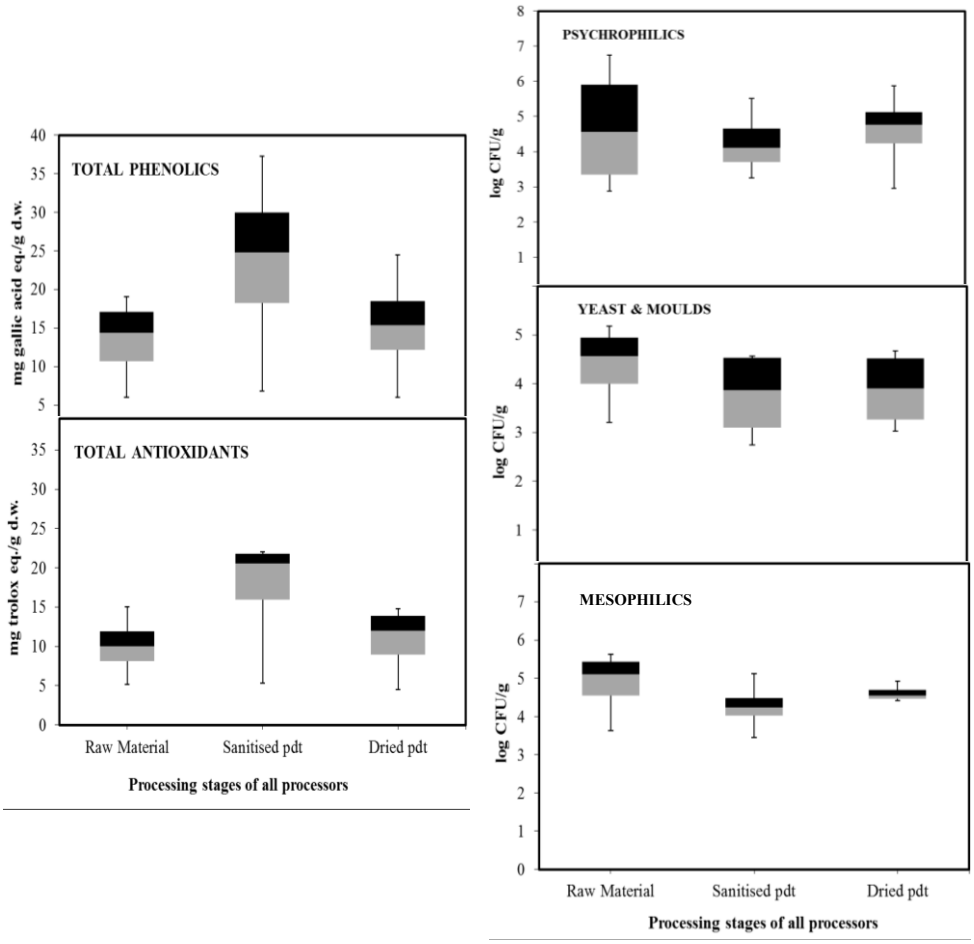


Figure 1.3: Boxplot showing total phenolic content and total antioxidant activity psychrophilic and mesophilic bacteria, and yeast and molds counts in log CFU/g in products from the four processors. The upper and lower boxes represent the quartiles (75th and 25th percentile), with the line separating the two boxes representing the median. The upper and lower whiskers show the highest and lowest values respectively, excluding outliers.

1.5.1.4 Effect of operation mode and processing steps of Processor A on quality of processed rocket leaves

In table 1.9 is reported the effect of processing steps (including a 2 days-storage at 5 °C) on quality attributes of minimally processed rocket leaves. Processing steps and storage did not significantly ($p \leq 0.05$) affect ascorbic acid content, total Vitamin C and total antioxidant activity. An increase of Dehydroascorbic acid, DHAA was only observed as a result of storage, whereas ascorbic acid did not change. Gas concentration after 2 days of storage evolved from 21 to 15.27 ± 0.12% of oxygen, showing an accumulation of CO_2 up to 4.87 ± 0.09%. These results are in line with what was reported by Amaro *et al*. (2015) which did not observe any reduction of AA during storage, and with the increase of DHAA reported by Falagán *et al*. (2015) during passive atmosphere storage at 5 ^{o} C, even if in the present study samples were stored only for 2 days, which is a very short period to observe significant oxidation.

Moreover there was an almost 81% rise in DHAA concentration compared to that of tunnel dried product (Table 1.9). Total phenolic, ascorbic acid and vitamin C compounds in addition to other phytochemicals contribute to plant antioxidant activity (Mukherjee *et al.*, 2013; Pinelo *et al.*, 2004). Among these, ascorbic acid is easily oxidized and gradually decreases during refrigerated storage and hence serve as a measure of processing effect on nutrient retention (Howard *et al*., 1999).

Though ANOVA did not show significant differences among processing steps, looking at the heat map distribution it can be observed that there were slight increases in Vitamin C and phenolic content after the 1st washing step which could be a response to stress on leaf surfaces impacted by the pressure from the direct bubbling action of the rocket leaves.

The combined physical and chemical effects of water bubbling and peroxyacetic acid (Table 1.1) may be comparable to that of exposing the product to negative and positive pressure conditions, resulting in the disruption of hydrophobic bonding on leaf surfaces, as suggested by Petri *et al*. (2015). Wounding stress has, in fact, been

reported to increase phenol content (Cisneros-Zevallos, 2003) and respiratory activity (Koltz *et al*., 2010; Escalona *et al*., 2015) in vegetables, but changes in total soluble and titratable acidity during the washing steps (data not shown) were not observed, probably because of low temperature conditions (Irtwange, 2006). Other authors also reported an increase of product respiration due to oxidative action of sanitizers (Vandekinderen *et al*., 2008). Since any phytonutrients reduction was observed for rocket leaves after washing, it can be concluded that the stress associated with the bubbling was more critical than the oxidative action of the sanitizer. Tunnel drying did not result in significant change in phytonutrients.

Bacteria counts, after being reduced by the sanitizing step, began to rise after drying and this could be attributed again to growth of returning epiphytic microorganisms (Turatti, 2015). Inversely the bubble action of the washing steps, enhanced contact between the leaves and the sanitizer, such that minimal processing of the rocket leaves resulted in a reduction of the microbial counts. Long *et al*. (2011) also reported the importance of bubbling effect for a good distribution of the sanitizer during washing. For Psychrophilic bacteria (PC) total reduction was of 1.24 log CFU/g after washing and only 0.87 log CFU/g after the drying tunnel. Yeast and moulds (Y&M) and Mesophilic bacteria counts (MC) reduced only by 0.65log CFU/g and 0.44 log CFU/g respectively in the final dried product (Table 1.9). Rocket leaves maintained close to neutral pH levels (data not shown) during the processing steps and storage with no significant differences. In addition, pH of washing water in the range of 7.0 -7.3 (Table 1.1), did not affect the sanitizing effect, since peroxyacetic acid is stable within a pH range of 1-8 (Artes *et al*., 2009).

Storage of packaged rocket leaves under passive atmosphere conditions showed no significant difference in ascorbic acid, total vitamin C and antioxidant content though dehydroascorbic acid significantly increase compared to the final tunnel dried product. Similarly, phenolic content remained almost stable during the storage period. Apart from Y&M which resulted in relatively significant increase of 0.99

log CFU/g, PC and APC did not change significantly during storage. Also other authors did not observe an effect of peroxyacetic in maintaining low microbial counts during passive modified atmosphere storage (Escalona *et al*., 2015).

Table 1.9: Effect of processing steps of Processors A on the quality characteristics of rocket leaves

PROCESSOR A								
Processing Steps	**Quality Characteristics**							
	AA mg/g d.w..	**DHAA mg/g d.w**	**Vit. C mg/g d.w.**	**TP mg gallic acid/g d.w**	**TAA mg trolox/ g d.w**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	13.89	0.95ab	14.84	5.99b	5.12	5.37	6.75	5.17ab
1st Washed pdt	16.18	0.85b	17.02	7.55a	5.60	5.06	5.68	4.52b
2nd Washed pdt	15.82	0.83b	16.65	6.84ab	5.29	5.12	5.51	4.57b
Tunnel dried product	11.87	0.67b	12.55	5.99b	4.46	4.93	5.88	4.67b
Storage at 5 degrees (2days)	12.34	1.21a	13.56	5.81b	5.01	5.14	6.59	5.66°
p-value	ns	***	Ns	**	ns	Ns	Ns	**

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: ns, ** and *** = not significant, significant at $P \leq 0.01$ and 0.001 respectively. (TP- Total phenol and TAA – Total antioxidant activity)

Volatile characterization and changes during minimal processing

A total of 18 volatile compounds were identified in rocket leaves belonging to the chemical groups of Aldehyde (5), Alcohols (3), Furans (2), Esters (1), Ketones (2), Sulphur and isothiocyanates (5) (Table 1.10). These compounds were similar to those reported in literature for minimal processing and storage (Rux *et al*., 2017; Spadafora *et al*., 2016; Mastrandrea, 2015). The volatile compounds in rocket leaves which were not influenced by the processing steps and storage ($p \leq 0.05$) were not included in the principal component analysis (PCA). Principal component analysis shows that 100% of the variance could be explained by four main components having eigenvalues greater than 1. Figure 1.4 shows the score plot (A)

and loading plot (B) of the variables in the PCA projected on the plane of first principal component PC1 vs the second principal component PC2, accounting for 75.31% of the variation observed.

The score plot (Figure 1.4A) shows a clear differentiation between the volatile compounds (Figure 1.4B) that were produced during the processing steps and passive atmosphere storage ein the multivariate space of the first two principal components.

Aroma and flavour volatiles contribute to consumer perception on the quality of a minimal processed product, however, minimal processing steps and post processing storage conditions may influence the flavour quality of rocket leaves. An understanding of the effect of processing steps on retention of the desirable volatiles and on the production of off-odor compounds will aid in improving operating conditions of processors. In this study, raw material rocket leaves were characterized by the highest concentration of (Z)-3-Hexen-1-ol, acetate (PC2) with leafy green vegetable aroma (Table 1.10), which has been reported in Brassica species (Charron and Sams, 1999). The emission of this compound in the raw material could be related to it's the plant defense signalling properties in response to fungal contamination (Shiojiri *et al*., 2006; Ameye *et al*., 2015). However, this green leaf aroma tended to decrease during the subsequent processing steps (Figure 1.4A and 1.4B). Minimal processing steps, and particularly the washing steps influenced the production of stress induced volatiles of rocket leaves, such that besides the retention of considerable amounts of (Z)-3-Hexen-1-ol and acetate, the 1st Washing induced the release of (Z)-2-Penten-1-ol which positively increased on the third principal component, PC3 (plot not presented), with a distinctive green aroma. Generally volatile alcohol compounds are synthesized through lipoxygenase (LOX) (Schreier and Schwab, 2002) and used by plants as defence mechanism (Ruther and Kleier, 2005). This could have been due to stress on the leaf surface membrane from bubble action of the washing water that has already been described to also influence phenol metabolism.

The alcohol produced during this 1st washing step could have contributed to the reduced concentration of total phenols after the 2nd washing step. Volatiles related to senescence began to increase after the second washing step (2nd W) which were characterised by 2-Methyl furan, Benzaldehyde, Dimethyl sulphide increasing on PC2 and (Z)-2-Penten-1-ol on PC3. Benzaldehyde has been reported to be an essential oil with an almond flavour that gives rocket leaves the sesame-seed like smell (Jirovetz *et al*., 2002). Furan compound evolution may have occurred due to membrane degradation of the rocket leaves during the washing steps as reported by Morini *et al.* (1995). Sulphur volatile has been found to be produced during secondary metabolism of amino acids (Belitz *et al.*, 2009), and this mainly as response to stress or as plant defensive mechanisms (Van Etten *et al.*, 2001; Rosenthal and Berenbaum, 1992). Hence stress during the washing activity may have resulted in some kind of degradation of cell membrane releasing the causal off-odour compound dimethyl sulphide (Jacobsson *et al*., 2004).

The Dried product, D had similar volatile compounds as the 2nd W but produced the highest concentration of 2-Methyl furan (PC2). It may be possible that 2-Methylfuran was produced starting from Vitamin C as precursor, since it was observed to decrease by 24.62%. Several studies have reported Vitamin C as an efficient precursor for furan production (Mark *et al*., 2006; Fan *et al*., 2008; Limacher *et al*., 2007; Owczarek-Fendor *et al*., 2012). The heat employed during the drying process may have influenced this effect, although its mechanism for the formation of 2-Methyl furan needs further investigation (Palmers *et al*., 2014).

Table 1.10: Effect of minimal processing steps and MAP storage of rocket leaves on volatile compounds (Processor A)

Aroma Volatile Classification	RT (min)	Processing steps	Aroma/Odor descriptors[a]
Aldehydes			
Hexanal	14.43	***	Fresh, grass, green, oil
(E)-2-Pentenal	16.65	****	Floral, green
(E)-2-Hexenal	20.86	***	Fat, floral, green grass, pungent
(E, E)-2,4-Heptadienal	33.45	***	Fat, nut, flower, plastic
Benzaldehyde	34.87	**	Bitter almond, malt, roasted pepper
Alcohols			
(Z)-2-Penten-1-ol	25.63	**	Green, plastic
(Z)-3-Hexen-1-ol	28.55	Ns	Green leaf, grass, herb
(Z)-2-Hexen-1-ol	29.49	Ns	Green leaf, wine
Furans			
2-Methyl furan[b]	7.66	**	Ethereal, acetone, chocolate
2-Ethyl furan	9.35	Ns	Butter, caramel
Esters			
(Z)-3-Hexen-1-ol, acetate	25.42	**	Fresh, leafy, floral, green vegetable
Ketones			
2-Butanone	7.76	Ns	Ether, fragrant, pleasant, sweet
1-Penten-3-one	11.85	****	Green, herb, metal, mustard, pungent
Sulphur compounds			
Carbon disulphide	4.92	Ns	Vegetable sulphide
Dimethyl sulphide	5.12	**	Cabbage, organic sulfur, wet earth
Methyl thiocyanate	23.72	****	Sulphur
Isothiocyanates			
n-Pentyl isothiocyanate[b]	33.12	*	Green
4-Methylpentyl isothiocyanate[b]	35.34	Ns	Pungent, horseradish

All volatile compounds were identified by comparing MS data to spectra from NIST library and previous work in our laboratory (Mastrandrea, 2015)
RT (min): Retention time in minutes; Significance: ns, *, **, *** and **** = not significant, significant at $P \leq 0.05$, 0.01, 0.001 and 0.0001respectively
[a]Odor descriptors were sourced from www.vcf-online.nl_EU-Flavislist
[b]Odor descriptors from published data Jirovetz *et al*. 2002; Sigma-Aldrich, 2000; referenced in Mastrandrea, 2015)

Compounds with the high or low concentration of odour threshold positively contribute to the overall odour of food (Alonso *et al*., 2009). In this case rocket leaves stored in passive atmosphere at 5 ^{o}C for 2 days, produced the highest amount of the off-odour volatile (E, E)-2, 4-Heptadienal, which is mainly formed through autoxidation of linolenic acid (Husain, 2010). Lipid autoxidation has been reported to cause significant changes to sensory properties including odour, flavour, colour and texture and consumer acceptance of food products (Jacobsen, 2010). Still, in the same lipid oxidation pathway, 1-Penten-3-one which has been described to have a pungent, rancid green smell increased (Genot *et al*., 2003). The activity of the lipase enzyme from the fungal infection that occurred by the yeast and molds (Table 1.9), might have released fatty acid from the product tissue and activated the lipoxygenase and hydroperoxide lyase pathways to convert linoleic and linolenic acid to Hexanal and *(E)*-2-Hexenal (Zeringue, 1995). The release of sulphur compounds (n-Pentyl isothiocyanate, Methyl thiocyanate) emitted as described by PC1, confirms the degradation activities due to membrane breakdown allowing the myrosinase enzyme to act on intact glucosinolates during storage (Agneta *et al*., 2013).

The heat map (Figure 1.5), provides a summary of the effects of the operation mode of processor A on the quality of rocket leaves, showing the changes that occurred in phytochemicals, aroma volatile groups and microbial quality during the minimal processing steps. It shows the relation among the quality variables in three main clusters groups; from the left, cluster 1: shows that phenols, antioxidants, AA and alcohols are related in their changes during processing and storage. Total phenols and antioxidants increased together as a result of the 1st washing step, while ascorbic acid and alcohols increased due to both the 1st washing and 2nd washing steps and decreased together after drying. After 2 days storage a slight increase in antioxidants and alcohols was observed. The observed increase in the phytochemical and alcohol during washing and storage confirms the effect of stress as already discussed.

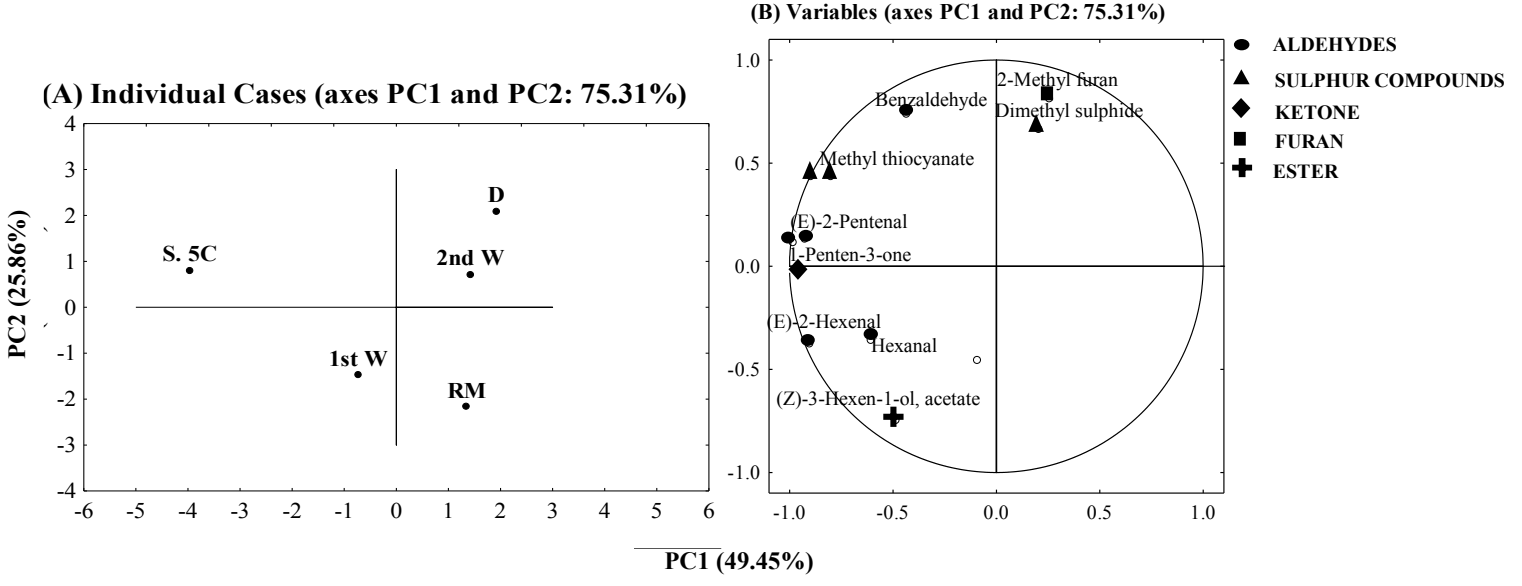


Figure 1.4: Principal Component analysis (PCA): Score plot for Raw material (RM), 1st Washed product (1st W), 2nd Washed product (2nd W), Dried product (D) and Stored product at 5°C (S. 5C) **(A)** and Correlation loading plot **(B)**. The volatile compounds are the mean of 3 replicates (n=3)

Cluster 2 and Cluster 3 are enough related, however Cluster 2 shows the relationship between microbial contamination and release of the ester volatile - (*Z*)-3-Hexen-1-ol, acetate, as already described, , all decreasing during the processing steps and increasing after 2days of storage. Cluster 3; shows the relation between DHAA and Ketones, Sulphur compounds and aldehydes. The ketone (1-Penten-3-one) characterized by pungent, spicy aroma, have also been observed in rocket leaves before and after MAP storage (Mastrandrea *et al*., 2017). In comparison to the raw material, and the 1st and 2nd washing steps, low levels of Ketones and DHAA were found in the tunnel dried product, which then increased during storage. Also high levels of sulphur compounds and aldehydes were detected during storage, this may be related to senescence and tissue degradation, since the volatiles are release during tissue degradation, while DHAA is produced as a result of oxidation of ascorbic acid.

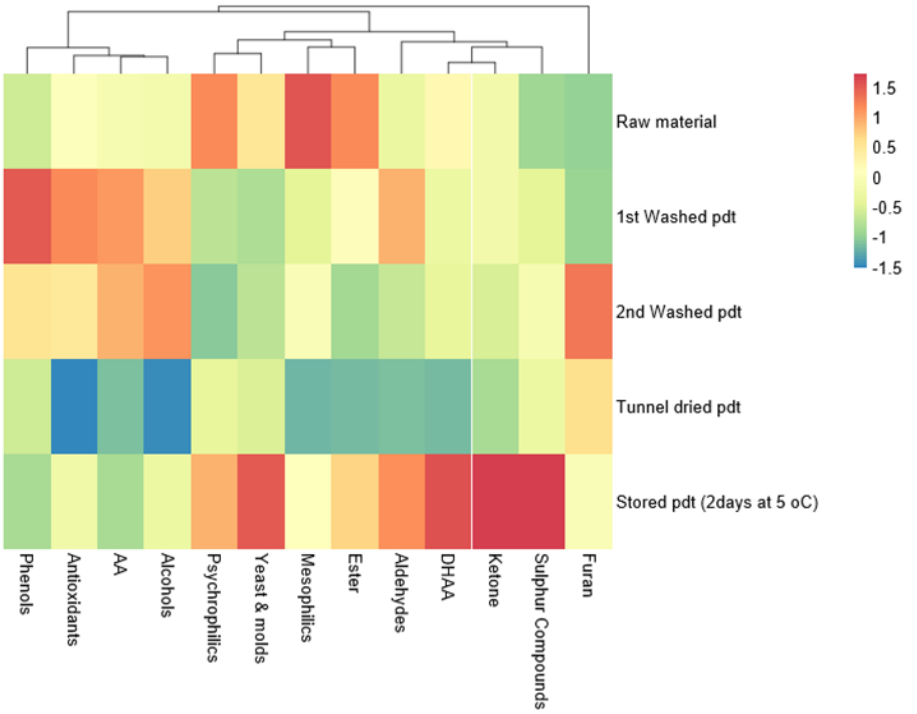


Figure 1.5. Cluster analysis and heat maps of phytochemical, volatile groups and microbial quality of rocket leaf as affected by operation mode and processing steps of processor A.

The last group were furans with low content in raw material which increased after the second wash but decreased during tunnel drying and storage, though emission levels after two (2 days) storage were higher than that of the raw material.

It can be concluded that the mode of operation of processor A was effective in maintaining phenols, though antioxidants and ascorbic acid content were lower in tunnel dried product compared to the initial values. It also showed reducing microbial counts, which increased in MAP storage. In regards to volatiles, with the exception of the washing steps which increased aldehydes and alcohols, most degradative volatiles were release during storage for 2days at 5^{o}C.

1.5.1.5 Effect of operation mode and processing steps of Processor B on quality of processed rocket leaves

For **Processor B** (Table 1.11), processing activities did not result in significant differences in total vitamin C (ascorbic acid, AA and dehydroascorbic acid, DHAA) and phenol content mg/g dry weight, except for total antioxidant content of rocket leaves which showed significant differences during those steps. Insignificant change in pH and titratable acidity (data not shown) may have aided in maintaining stable to slight increases in vitamin C content during processing (Pastori and Foyer 2002). The pH and titratable acidity of the rocket leaves during processing and storage did not significantly change (data not shown). Storage conditions did not result in significant differences in phytochemical content (AA, DHAA, Vit. C and total phenols) between the final dried product and stored product.

Microbial counts in log CFU/g for PC, Y&M and MC decreased during processing activities but without significant differences. Slight increases in microbial count after drying observed in this study has also been reported by Martinez-Sanchez *et al*. (2006). MC remained almost stable in storage at 5 °C for 5 days but PC and Y&M increased significantly by 2.29 log CFU/g and 1.13 log CFU/g respectively (Table 1.11). The quality of minimal processed leaves with regards to total vitamin C, ascorbic acid, dehydroascorbic acid, total phenols and antioxidant activity were retained in the final dried product after minimal processing. Stress during the 2nd Washing step led to slight increases in antioxidant activity (Table 1.11) probably due to tissue shock as reported by Martinez-Sanchez *et al*. (2008). No other significant changes in phytochemical properties (Table 1.11) and volatile properties (Table 1.12) was observed, may be because of the less oxidizing effect of the sanitizer used by this processor and of the low temperature of washing water (Table 1.2) reducing respiratory activities of the rocket leaves. Storage at 5^{o}C in passive modified atmosphere did not affect rocket leaf nutritional and phytochemical properties but increased microbial load although they were all below the limit for consumption minimal processed vegetables.

Table 1.11: Effect of processing steps of Processors B on the quality characteristics of rocket leaves

	PROCESSOR B							
Processing Steps	**Quality Characteristics**							
	AA mg/g dry wgt.	**DHAA mg/g dry wgt.**	**Vit. C mg/g dry wgt.**	**TP mg gallic acid/g dry wgt**	**TAA mg trolox/g dry wgt**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	12.43	1.28	13.71	16.45	14.99ab	4.85	3.51b	4.87ab
Pre-Washed pdt	11.36	1.11	12.47	17.22	13.72b	4.69	3.58b	4.85b
1st Washed pdt	13.62	1.38	15.00	19.66	16.20ab	4.97	3.24b	4.59b
2nd Washed pdt	15.37	1.37	16.75	22.00	19.46a	4.26	3.25b	4.51b
Tunnel dried product	11.52	1.21	12.73	16.46	13.57b	4.48	2.96b	4.46b
Storage at 5 degrees (5 days)	11.70	1.06	12.76	16.22	17.67ab	4.97	5.25a	5.59a
p-value	ns	ns	Ns	Ns	*	Ns	**	***

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: ns, *, ** and *** = not significant, significant at $P \leq 0.05$, 0.01 and 0.001 respectively. (TP- Total phenol and TAA – Total antioxidant activity)

Volatile characterization and changes during minimal processing

This experiment confirmed the identification of the same volatiles, found for processor A; a total of 20 volatile compounds were identified in rocket leaves after the processing steps and passive modified atmosphere (MAP) storage at 5^{o}C for 5days, belonging to the chemical groups of Aldehyde (5), Alcohols (4), Furans (2), Esters (1), Ketones (2), Sulphur and isothiocyanates (6) (Table 1.12). The Principal component analysis, including only volatiles showing significant differences with processing steps, shows that 77.81% of the variance could be explained by two main components having eigenvalues greater than 1. Figure 1.6 shows the score (A) and loading plot (B) of the variables in the PCA projected on the plane of first 2 principal components (PC1 and PC2).

The raw material had the highest concentration of 4-Methylpentyl isothiocyanate which varied together on the second principal component (PC2), and decreased during the processing steps and after storage of 5days at 5^{o}C. This could be attributed to the volatility and susceptibility of isothiocyanates to hydrolysis (Shapiro *et al*., 1998), since the minimal processing steps did not seem to have influenced the production of degradative volatiles. Sample after 5 days of storage in passive atmosphere resulted in increased concentration of off-odour volatiles as Benzaldehyde, Methyl thiocyanate and Dimethyl sulphide (Figure 1.6A and 1.6B). Benzaldehyde has been reported in fermented Brassica products (Zhao *et al*., 2007), whereas the production of Sulphur compounds (Methyl thiocyanate and Dimethyl sulphide) during storage of rocket leaves have been reported by other authors (Spadafora *et al*., 2006; Mastrandrea *et al*., 2017; Luca *et al*., 2016; Neilson *et al*., 2008). Dimethyl sulphide has been reported to have a distinctive smell of 'rotten cabbage' and its production could have been due to the increase in psychrophilic bacteria as observed during storage (Nielsen *et al*., 2008). Increase in microbial load especially psychrophilic bacteria may have caused the increase in dimethyl sulphide (off-odor compound) in storage.

The heat map (Figure 1.7), shows the effect of the operation mode on the changes of phytochemicals, volatile groups and microbial quality of rocket leaves during the processing steps of processor B. Clusters show that phenol and ascorbic acids were well related, though DHAA also responded similarly to processing steps. Phenols, AA, DHAA increased as a result of the 1st washing and the 2nd washing steps and decreased in the tunnel dried and stored products at 5^{o}C. Though antioxidant activity also increased as a result of the 1st and 2nd washing steps, contrary to phenols and Vit C, it also increased in storage, together with the increase of psychrophilic bacteria counts. Figure 1.7 also shows the relationship among yeast and molds, sulphur compounds and aldehydes (benzaldehyde). It depicts, that yeast and mould levels in raw material and pre-washed product, decreased as a result of the washing steps and the tunnel dried product together with the decrease of the volatiles. Moreover, after 5 days storage at 5^{o}C, increased yeast and mold counts

influences the increased production of sulphur compounds and aldehydes suggesting the development of off-odor.

Table 1.12: Effect of minimal processing steps and MAP storage of rocket leaves on volatile compounds (Processor B)

Aroma Volatile Classification	RT (min)	Processing steps	Aroma/Odor descriptors[a]
Aldehydes			
Hexanal	14.43	ns	Fresh, grass, green, oil
(E)-2-Pentenal	16.65	ns	Floral, green
(E)-2-Hexenal	20.86	ns	Fat, green grass, pungent
(E, E)-2,4-Heptadienal	33.45	ns	Fat, nut, flower, plastic
Benzaldehyde	34.87	**	Bitter almond, malt, roasted pepper
Alcohols			
1-Pentanol	22.28	ns	Balsamic, green, yeast
(Z)-2-Penten-1-ol	25.63	ns	Green, plastic
(Z)-3-Hexen-1-ol	28.55	ns	Green leaf, grass, herb
(Z)-2-Hexen-1-ol	29.49	ns	Green leaf, wine
Furans			
2-Methyl furan[b]	7.66	ns	Ethereal, acetone, chocolate
2-Ethyl furan	9.35	ns	Butter, caramel
Esters			
(Z)-3-Hexen-1-ol, acetate	25.42	ns	Floral, green
Ketones			
2-Butanone	7.76	ns	Ether, fragrant, pleasant, sweet
1-Penten-3-one	11.85	ns	Green, herb, metal, mustard, pungent
Sulphur compounds			
Carbon disulphide	4.92	ns	Vegetable sulphide
Dimethyl sulphide	5.12	****	Cabbage, organic sulfur, wet earth
Methyl thiocyanate	23.72	****	Sulphur
Isothiocyanates			
Methyl isothiocyanate	22.03	ns	Mustard oil
n-Pentyl isothiocyanate[b]	33.12	ns	Green
4-Methylpentyl isothiocyanate[b]	35.34	*	Pungent, horseradish

All volatile compounds were identified by comparing MS data to spectra from NIST library, work done by Blažević and Mastelić, 2008 and previous work in our laboratory on rocket leaves (Mastrandrea, 2015)
RT (min): Retention time in minutes
Significance: ns, *, **, *** and **** = not significant, significant at $P \leq 0.05$, 0.01, 0.001 and 0.0001, respectively
[a]Odor descriptors were sourced from www.vcf-online.nl_EU-Flavislist
[b]Odor descriptors from published data Jirovetz *et al*. 2002; Sigma-Aldrich, 200; referenced in Mastrandrea, 2015)

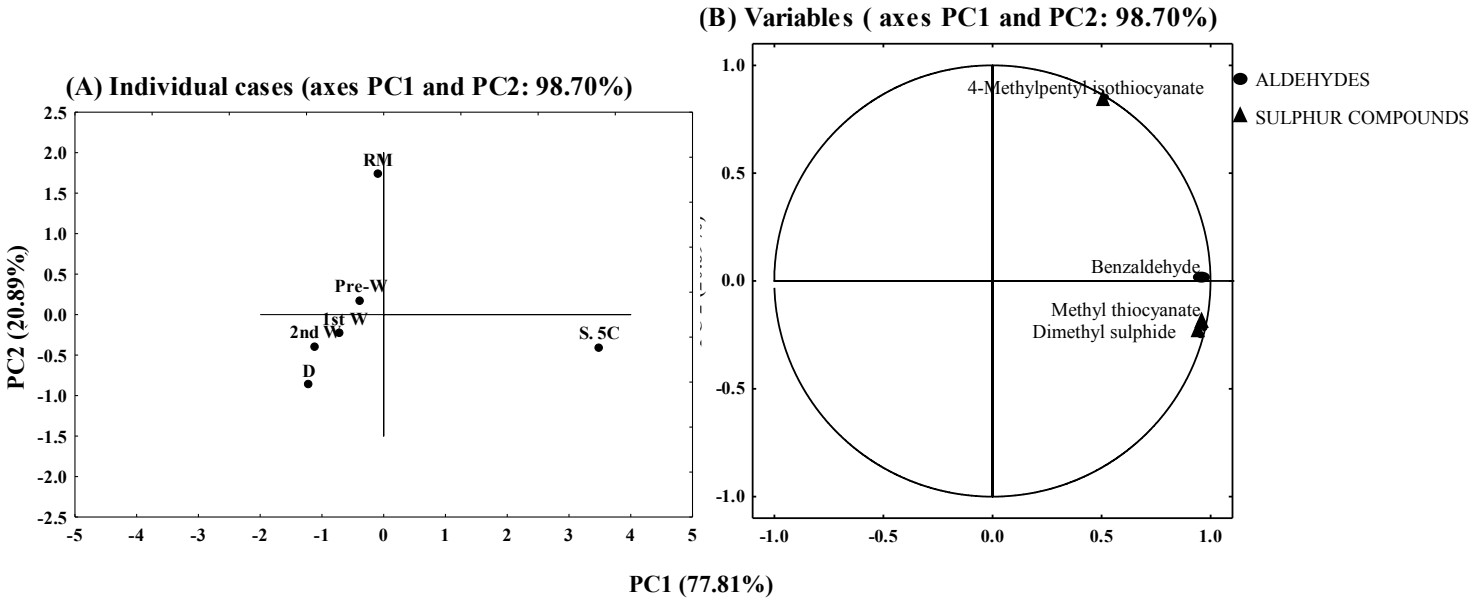


Figure 1.6 : Principal Component analysis (PCA): Score plot for Raw material (RM), 1st Washed product (1st W), 2nd Washed product (2nd W), Dried product (tunnel dried) (D) and Stored product at 5ºC (S. 5C) **(A)** and Correlation loading plot **(B)** describing two volatile organic group of compounds and their changes during minimal processing steps of rocket leaves. The volatile compounds as indicated are triplicate sample means
(n=3)

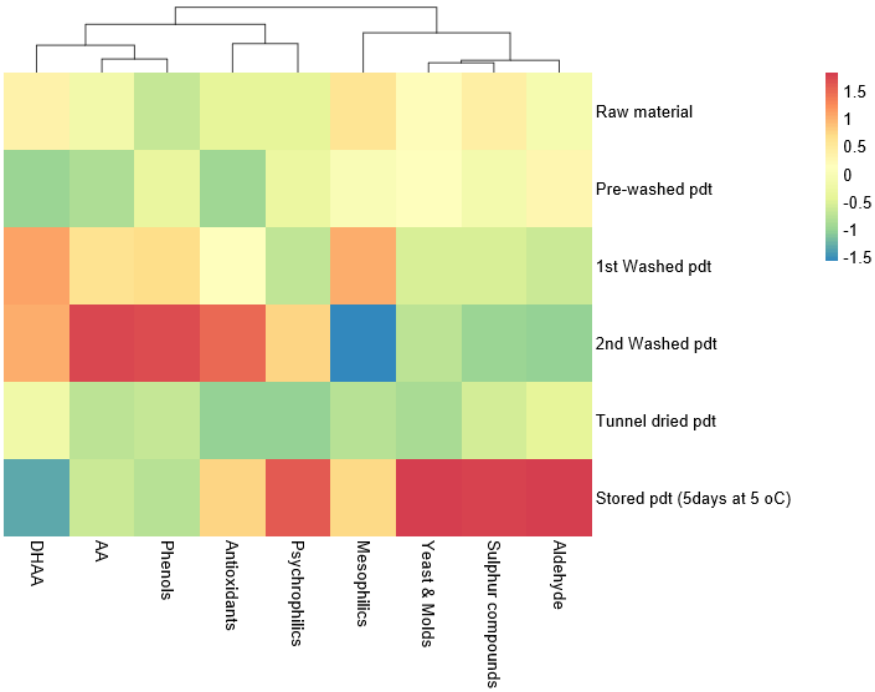


Figure 1.7: Cluster analysis and heat maps of phytochemical, volatile groups and microbial quality of rocket leaf as affected by operation mode and processing steps of processor B

Though the mode of operation of processor B was different from Processor A, the changes in the phytochemical, nutritional and microbial quality of rocket leaves during the washing steps were similar. In conclusion, the mode of operation of processor B did not cause substantial changes of quality of rocket leaves, suggesting less stress compared to that of processor A, except for the increase of antioxidant activity as a result of the washing step. Degradative volatiles were produced only after storage at 5^{o}C for 5 days, due to increase in microbial counts.

1.5.1.6 Effect of operation mode and processing steps of Processor C on quality of rocket leaves

In case of Processor C, processing steps affected many quality attributes of rocket leaves, As observed in the rocket leaves analysed from Processor A rocket leaves from **Processor C** (Table 1.13) also had significantly increased levels of total phenols by 18.27 mg gallic acid/g dry wgt) and antioxidant activity by 12.96 mg trolox /g dry wgt after the washing steps which were reduced after spin drying. Total phenol and antioxidant content increased after the washing and sanitizing step and although reduced reduction was induced by the spin drying, their content at the end of the processing line was higher than the raw material. This result was different from what was observed from processors A and B and this may be due to the different systems used for drying, which was a spin dryer in this case. The heat used during tunnel drying for processor A and B could have contributed to the final reduction of antioxidants. Increase in phenolic acids (Sinapic and Ferulic acid) during the washing steps may have led to increased phenolic and antioxidants in washed product. Rocket raw material contained 0.004mg/g sinapic acid (Figure 1.8 A) and 0.0039 mg/g Ferulic acid (Figure 1.8 B) on dry weight basis.

During minimal processing, the sinapic acid and ferulic acid content began to increase after pre-washing and peaked to the highest content (0.06 mg/g dry weight) for sinapic acid after the 2nd Wash. For Ferulic acid, the highest peak was observed after the 1st Washing step (0.022 mg/g dry weight). Both Sinapic acid and ferulic acid content reduced in the rocket leaves after spin drying to 0.02mg/g d.w. and 0.01mg/g d.w. respectively. It was noticed that, though spin drying reduced the content of sinapic and ferulic acids in rocket leaves, compared to that of the washing steps, their levels were still higher than that of their respective raw material (Figure 1.8 A and 1.8 B; Figure 1.10), as also described for total phenols (Table 1.13). Commercial peroxyacetic acid, Tsunami as used by processor C have been reported to maintain sensory quality of rocket leaves with no damaging effect on antioxidant constituents (Martínez-Sánchez *et al*., 2006).

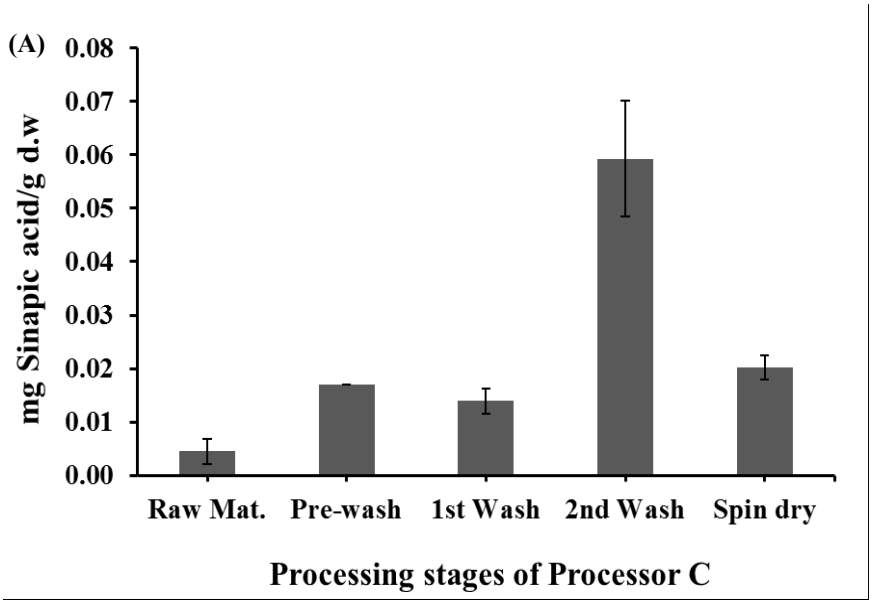
Raw material microbial counts were very low, however washing operations were not effective in reducing, microbial population, with a significant increase of 1.78 log CFU/g (PC) and 0.78 log CFU/g (MC) observed after drying. Though the washing steps showed decreased Y&M counts, no significant differences were observed throughout the processing steps.

It was observed that the sanitization step was effective in reducing microbial counts of only for the yeast and molds and not for psychrophilic and mesophilic bacteria. Although there was a drop in microbial load for MC as well as yeast and moulds, processing steps resulted in increased microbial load which was even augmented after spin drying, probably due to cross-contamination from recycled water or a small part of contaminated rocket leaves in a batch as reported from other authors (Allende *et al*., 2008; Gil *et al*. 2009).

Table 1.13: Effect of processing steps of Processors C on the quality characteristics of rocket leaves

	PROCESSOR C				
Processing Steps	**Quality Characteristics**				
	Phenols mg gallic acid/g dry wgt	**Antioxidant mg trolox/g dry wgt**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	19.03c	9.09c	3.63b	2.88b	3.20
Pre-Washed pdt	28.99b	15.08bc	3.07c	2.82b	2.59
1st Washed pdt	26.62b	18.60ab	3.05c	3.00b	2.61
2nd Washed pdt	37.30°	22.05a	3.45bc	3.86ab	2.75
Spin dried product	24.43bc	14.78bc	4.41a	4.66a	3.03
p-value	***	***	****	**	ns

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: ns, **, ***and **** = not significant, significant at $P \leq 0.01$, 0.001 and 0.0001 respectively



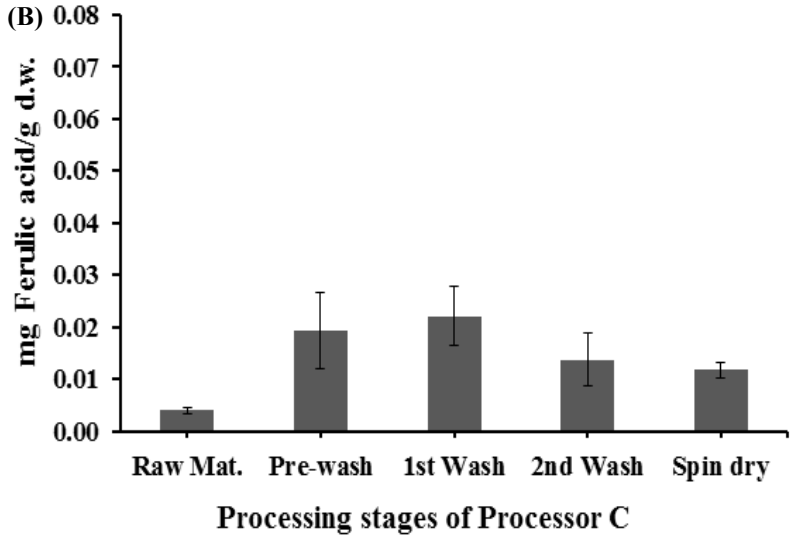


Figure 1.8: Changes in phytochemical content: (A) Sinapic acid and (B) Ferulic acid during each of the minimal processing steps of Processor C

Total Bacteria Screening assay was carried out to confirm the results of microbial counts (Table 1.14) using RT-PCR. As routinely used in other studies, 5µL reaction mixture was used in the RT-PCR. A fluorescent probe was used to monitor the amount of product at the end of each cycle and the real-time PCR instrument expressed the cycle in which it first detected fluorescence. The greater the number of starting copies (bacterial DNA template), the fewer cycles required to achieve fluorescence detection. The results (Figure 1.9) showed similar microbial counts as observed in Table 1.13. The recorded increase in sample fluorescence above the established baseline value of 15, is proportional to the amount of the accumulated PCR product during the processing steps, and as shown occurred after many cycles. Table 1.14, shows high fluorescence threshold cycle values for raw material rocket leaves which decreased during the processing steps, explaining that the lower bacteria count observed in the raw material which increased during the processing steps was probably due to cross-contamination as already reported.

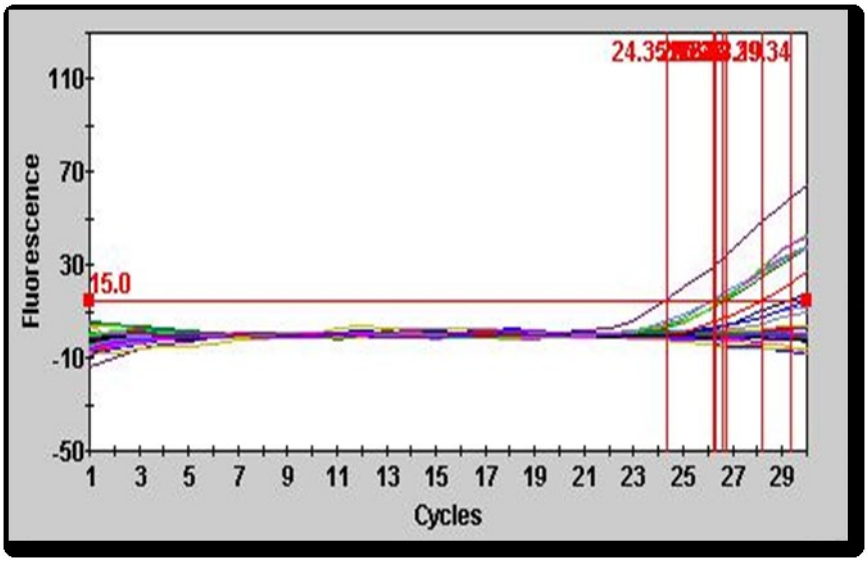


Figure 1.9: Detection cycles of total bacteria during the minimal processing steps of Processor C

Table 1.14: Mean FAM Ct values for Total Bacteria Screening Assay

Processing Steps	RT-PCR Results	
	TBS (FAM)	**FAM Ct***
Raw Material	+	29.34
Pre-Washed pdt	+	26.76
1st Washed pdt	+	27.40
2nd Washed pdt	+	26.27
Spin dried product	+	24.35
Negative control	-	0

*FAM Ct (fluorescence threshold cycle value based on a fluorescein dye)
Low FAM Ct value represents high microbial counts and vice versa

The heat map (Figure 1.10) illustrates the effects of mode of operation of Processor C on changes in phytochemicals and microbial counts during the minimal processing steps of rocket leaf products. From the left: Cluster 1: It depicts reduction in mesophilic, psychrophilic counts and yeast and molds of raw material during the prewashing step which began to rise again after the 1st washing (PC) or 2nd washing step, (MC and Y&M) reaching the highest counts after drying suggesting cross-contamination as described. Cluster 2; shows changes in phytochemicals (antioxidants, phenol and phenolic acids - sinapic acid and ferulic acid) increasing from raw material (lowest content) during the processing steps to high levels after the 2nd washing step except than ferulic (maximum peak after 1st washing) and decreased in the spin dried product. The phytochemical content in the dried product was higher than that of than of the raw material prior to processing.

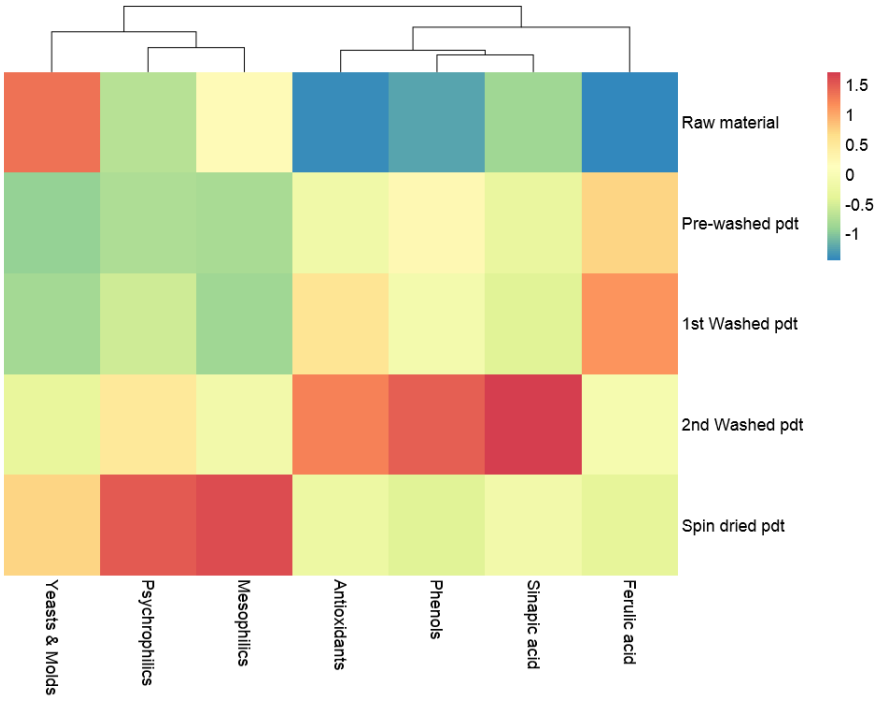


Figure 1.10: Cluster analysis and heat maps of phytochemical and microbial quality of rocket leaf as affected by operation mode and processing steps of processor C.

It can be concluded that the mode of operation for processor C improved phytochemical properties of rocket leaves and also decreased microbial counts to some extent, but for possible cross-contamination that occurred during processing steps, an increase in microbial counts after spin drying was observed.

1.5.1.7 Effect of operation mode and processing steps of Processor D on quality of rocket leaves

For **Processor D** anova results are reported in Table 1.15. It can be observed that, phenol content and antioxidant activity increased significantly during the washing steps, but there were no significant differences between the total phenol content and antioxidant activity of the raw material and the spin dried product. After the spin drying step, the level of phenol and antioxidants were reduced by about 50%.

Microbial counts of MC, PC and Y&M log CFU/g was reduced after sanitization step, whereas a slight increase in microbial counts for MC and PC was observed after the spin drying step. Nonetheless, the final reduction of MC, PC, Y&M counts from the raw material to the dried product were 1.01 log CFU/g, 0.76 log CFU/g and 0.93 log CFU/g, respectively.

Rocket leaf product quality from this processor was similarly affected by minimal processing steps, as with the other processors. Response of Sinapic acid and Ferulic acid content of rocket leaves to minimal processing steps and operating modes of this processor were consistent with that of processor C.

Table 1.15: Effect of processing steps of Processors D on the quality characteristics of rocket leaves

	PROCESSOR D				
Processing Steps	**Quality Characteristics**				
	Phenols mg gallic acid/g dry wgt	**Antioxidant mg trolox/g dry wgt**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	12.20c	10.90c	5.62a	5.62a	4.27a
Pre-Washed pdt	21.23b	11.86bc	4.97b	4.89b	3.05b
1st Washed pdt	21.93b	19.93ab	3.80e	3.88d	3.08b
2nd Washed pdt	27.53a	21.66a	4.21d	4.37c	3.22b
Spin dried product	14.16c	10.46c	4.61c	4.86b	3.34b
p-value	****	**	****	****	***

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: **, ***and **** = significant at $P \leq 0.01$, 0.001 and 0.0001 respectively.

Raw material content of Sinapic acid and Ferulic acid prior to minimal processing were 0.15 and 0.01mg/g dry weight respectively (Figure 1.11 A and 1.11 B). Similar to that of Processor C, washing and sanitizing steps increased sinapic acid and ferulic acid content in the rocket leaves significantly with a peak of 0.52mg/g dry weight for sinapic acid after the 2nd Wash (Figure 1.11 A) and a peak of 0.08 mg/g dry weight for ferulic acid after the 1st Wash step (Figure 1.11 B). Spin drying reduced the content of both Sinapic acid and Ferulic acid significantly after washing to 0.08 and 0.01mg/g dry weight respectively.

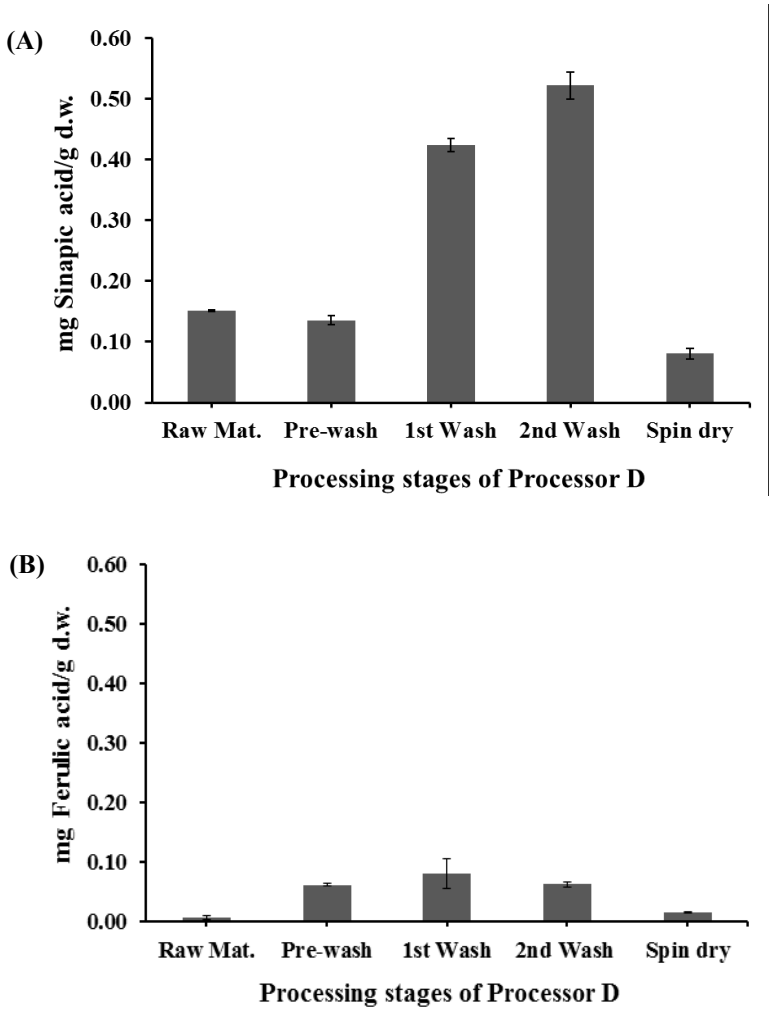


Figure 1.11: Changes in phytochemical content: (A) Sinapic acid and (B) Ferulic acid during each of the minimal processing steps of Processor D

Total Bacteria Screening assay was carried out on samples from Processor D to confirm the results of bacteria counts (Table 1.16) using RT-PCR. The results showed similar microbial counts as observed in Table 1.15. Table 1.16, shows low fluorescence threshold cycle values for raw material rocket leaves which increased during the processing steps, indicating that higher bacteria count, observed in the raw material, decreased during the processing steps.

Table 1.16: FAM Ct values for Total Bacteria Screening Assay

Processing Steps	**RT-PCR Results**	
	TBS (FAM)	**FAM Ct***
Raw Material	+	22.86
Pre-Washed pdt	+	26.34
1st Washed pdt	+	29.21
2nd Washed pdt	+	26.84
Spin dried product	-	0
Negative control	-	0

*FAM Ct (fluorescence threshold cycle value based on a fluorescein dye)
Low FAM Ct value represents high microbial counts and vice versa

In particular from Figure 1.12, we may observe that although washing steps reduced microbial counts (MC, PC and Y&M) the subsequent dried product showed a slight increase, but that the final load was lower than that of the raw material prior processing. On the other hand, increased total phenol content and antioxidant activity during the washing steps, did not affect the final spin dried product and resulted in rocket leaf products which had phytochemical content similar to that of their initial raw material, as for processor A and B.

Figure 1.12 shows the 2 main clusters in the heat map. From the left: Cluster 1 depicts similar changes in phytochemicals during the processing steps, some differences were observed. Antioxidant activity and sinapic acid content of raw material, increased similarly during the 1st and 2nd washing activity but decreased after the spin drying; in the case of phenol and ferulic acid content., relatively low amount in the raw material, increased as a result of the prewashing, 1st washing and

2nd washing steps but decreased in the spin dried products. As for cluster 2, Mesophilic and psychrophilic bacteria counts, and yeast and molds, decreased after the 1st washing step but begin to rise after the 2nd washing and also in the spin dried product. Despite this increase, the levels of microbial counts were lower in the final product than the raw material.

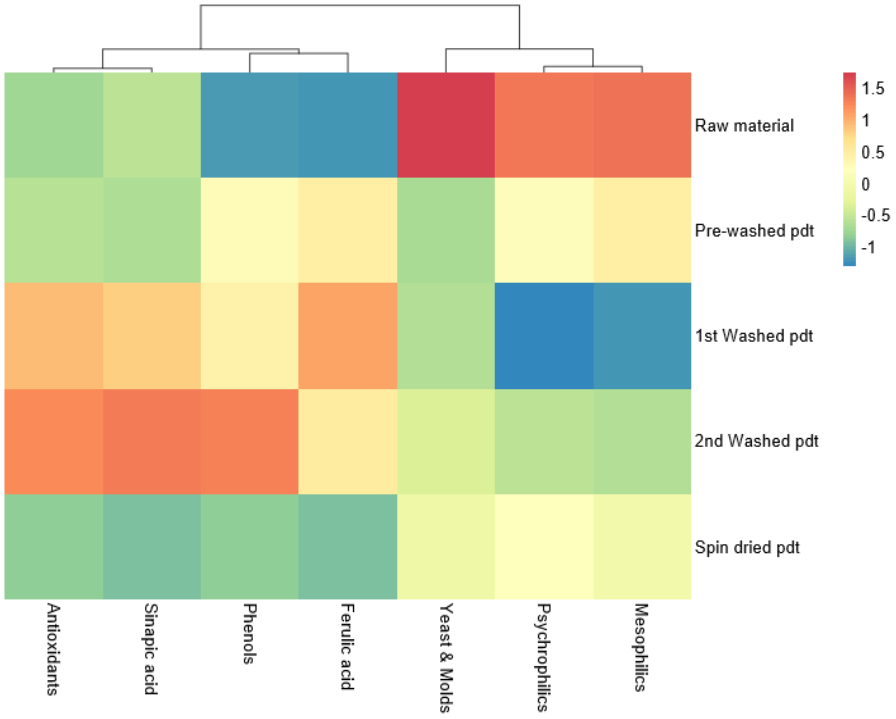


Figure 1.12: Cluster analysis and heat maps of phytochemical and microbial quality of rocket leaf as affected by operation mode and processing steps of processor D.

The mode of operation of processor D, increased phytochemical during the washing steps to high levels which were not maintained in the spin dried product with the exception phenols and ferulic acids which after processing were higher than those of the raw material. The operations during the minimal processing steps reduced microbial counts in the final product.

1.5.2. Lettuces

1.5.2.1 Raw material quality

Main quality attributes of Romaine and Iceberg lettuces used for these experiments are shown in Table 1.17. Romaine lettuce is known to have higher phytochemical properties like phenols than iceberg lettuce (Cantwell and Suslow, 2002b), but the initial raw material used in this study showed different results. Significantly lower phytochemicals (55% less phenols and 70% less antioxidant activity) were found in Romaine lettuce compared to iceberg lettuce (Table 1.17). Lower phytochemicals (total phenol and antioxidant activity) observed in Romaine lettuce as compared to iceberg lettuce could be due the pre-harvest factors like different cultivation times, location, light, humidity, nutrient absorption, physiological step, harvest times as well as postharvest temperature fluctuations (Viacava *et al*., 2014; Howard *et al*., 2012). Nonetheless, majority of lettuce leaf tissues contain little quantities of phenolic compounds when the plants are grown under conditions which do not impact stress (Tomas-Barberan *et al*., 1997) and also low levels of Vitamin C content in lettuce has been reported (López-Gálvez *et al*., 2010; Cantwell *et al*., 2016)

Vegetables like lettuce are very sensitive to microbial contamination as they have almost close to neutral pH levels (Parish *et al*., 2003). As reported by Beuchat (1996), majority of the fresh produce reaching the industry had microbial populations from 4 to 6 log CFU/g. Initial raw material of iceberg and romaine lettuce had microbial loads (Table 1.17) below the limit of 8 log CFU/g for fresh vegetables (Debevere, 1996; Allende *et al*., 2004), but yeast and molds count for iceberg lettuce was above the recommended limit of 5 log CFU/g (Debevere, 1996; Jacxsens *et al*., 1999; Oms-Oliu *et al*., 2008). Microbial counts may be attributable to harvest and postharvest handling, including transport, distribution and pre-storage environments; higher level observed in lettuces compared to rocket leaves may be due to the fact that lettuces were produced in open fields, and rocket in green houses.

Table 1.17: Quality characteristics of raw materials (lettuce) sampled prior to minimal processing

Processor	Product (lettuce)	Phenols mg gallic acid/g dry wgt	Antioxidant mg trolox/g dry wgt	Mesophilic Count log CFU/g	Psychrophilic bacteria log CFU/g	Yeast and Molds log CFU/g
A	Iceberg	6.21±0.34a	7.40±0.13a	6.20± 0.28a	5.93 ± 1.08a	6.15 ±0.67a
B	Romaine	2.80±1.20b	2.21± 0.78b	5.30± 0.13b	5.27 ± 0.27a	4.60± 0.57b

Values are means ± standard deviation of triplicate samples (n=3). Values not followed by the same letters in columns are not significantly different, while values followed by different letters in a column are significantly different at ($P<0.05$)

1.5.2.2 Effect of operation mode and processing steps on quality of fresh-cut Iceberg Lettuce

Minimal processing steps of fresh-cut iceberg lettuce may have caused physiological and oxidative stress to the final product. The stress exerted on the tissues led to depletion of defense phytonutrients (Reyes *et al*., 2007). Total vitamin C, ascorbic acid, total phenols and antioxidant activity decreased significantly during the minimal processing steps, but there were no significant changes in dehydroascorbic acid content (Table 1.18; Figure 1.13).

Although total vitamin C content was initially low (1.78 mg/g dry weight) in iceberg lettuce, the coring and cutting step initiated losses in total vitamin C content by 21 %, with the maximum loss occurring after the first wash. The results show that more than half (50 %) of the vitamin C content in the raw material iceberg lettuce was lost after the first washing step. Further processing steps; second washing and drying did not result in significant differences ($p<0.05$) in total vitamin C which was almost stable after the first washing step. Vitamin C is usually used as an index of the nutritional quality of food due to its unstable nature (Lee and Kader, 2000; Rojas and Gerschenson, 2001). In addition, about 50 % of both phenols content (initial value of 6.21 mg gallic /g d.w) and antioxidant activity (7.40 mg trolox/g d.w in the raw material) were also lost during the minimal processing operation (Table 1.18; Figure 1.13), which may have occurred due to oxidation of pre-existing phenols and total antioxidant due to cutting injury (Fleuriet and Macheix, 2003). Phenol compounds retard oxidative degradation of lipids and

contributes to improving nutritional value and quality of products (Aberoumand and Deokule, 2010). Also the antioxidant activity, was reduced by about 50 % after drying, in accord with Vitamin C and phenol content (Table 1.18).

As regarding to storage in MAP at 5 °C, by the third day a greater proportion (1.13 mg/g d.w.) of the total Vitamin C of the raw material prior to processing (1.78mg/g d.w) was lost; storage alone led to about (35 %) of this amount. At this time gas concentration within packaging were 2.5% to 0.75% O_2 and 7% to 8.85% CO_2 (data not shown)- As observed by other authors, loss of vitamin C content after 3 days storage was mainly due to the ascorbic acid reduction (Beltrán *et al*., 2005; Gil *et al*., 1999). Contrary to the Vitamin C content in storage, total phenols and antioxidant activity of the packaged product after 3 days increased by 74% and 36% respectively, resulting in a total loss from initial raw material of 17% and 36% for phenol content and antioxidant activity, respectively. Increased antioxidant activity of iceberg lettuce in storage was therefore more related to phenolic compounds than vitamin C. Increase of antioxidant during storage have been reported in other ready to eat products (Artes *et al*., 2008), as result of stress occurred during processing. Results show that after 3 days in MAP storage at 5^{o}C, pH (7.1), titratable acidity (0.17% malic acid) and total soluble sugar content (3) did not change (data not shown) from that of the final dried product prior to packaging.

Regarding microbial counts, washing and sanitizing is a critical part of fresh-cut processing prior to drying and packaging. It is the only step in which microbial load of raw slice product may be reduced. Microbial load on raw material reduced by about 46% after the 1st washing for the bacteria groups and for yeast and molds analyzed in this study (Table 1.18; Figure 1.13). Similar to the results of this study, microbial reductions of 1-2 log units were achieved after washing in other studies (Akbas and Ölmez, 2007; Ölmez and Kretzschmar, 2009; López-Gálvez *et al*., 2010). This may have been achieved as a result of the bubbling effect of washing and sanitizing treatment. In addition, the washing stages rinse any solutes/cellular fluids released after cutting which act as nutrient for micro-organisms to proliferate

(Toivonen and Stan 2004; Qadri *et al*., 2015). Regarding the drying step, despite the slight increase observed, dried product showed significantly reduced psychrophilic bacteria, yeast and mold count as well as mesophilic bacteria by 2.15 log CFU/g, 2.24 log CFU/g, 2.54 log CFU/g with respective to the raw material (Table 1.18).

Microbial quality of fresh-cut products in active MAP storage is dependent on microbial load on the surface of the product prior to storage. This is because, although low O_2 conditions in packages delay microbial proliferation, they are unable to halt growth even under refrigerated conditions (Aguayo *et al*., 2001; Zagory, 1999). Hence, the slight but not substantial increases of 15 %, 9 % and 8 % for mesophilic bacteria, psychrophilic bacteria and yeast and molds recorded after 3 days of storage in active MAP storage.

On the whole, there was 30% reduction in all microbial groups analyzed, even after 3 days of storage compared to their corresponding raw material prior to processing (Table 1.18; Figure 1.13).

Figure 1.13 shows the 3 main clusters of the variables (columns) in the heat map and two main clusters (rows) of the processing steps. From the left (columns): Cluster 1 shows close relationship between phenols and antioxidant activity which decreased together during the processing steps as shown to the lowest concentration in the dried product. However after 3 days storage their concentration increased again but was still lower than that of the initial raw material. Similarly in Cluster 2: AA and DHAA, PC, Y&M and MC decreased together during the processing steps but while AA and DHAA decreased further after 3 days of storage, PC, Y&M and MC increased after 3days of storage. Nonetheless, alcohol levels increased after coring and cutting, with decreasing phytochemicals but generally decreased during the subsequent processing steps and increased again after 3days of storage, signifying stress responses during the coring and cutting step and storage for 3days.

Table 1.18: Effects of processing steps of Processors A on the quality characteristics of fresh-cut Iceberg lettuce

PROCESSOR A								
Processing Steps	**Quality Characteristics**							
	AA mg/g dry wgt.	**DHAA mg/g dry wgt.**	**Vit. C mg/g dry wgt.**	**Phenols mg gallic acid/g dry wgt**	**Antioxidant mg trolox/g dry wgt**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	1.05a	0.73	1.78a	6.21a	7.40°	6.20a	5.93a	6.15a
Cored &quartered pdt.	0.87ab	0.61	1.47ab	5.39a	6.26ab	5.68a	4.96ab	5.23a
1st Washed pdt	0.45c	0.37	0.83bc	3.83bc	5.02ab	3.35c	3.19c	3.28c
2nd Washed pdt	0.50bc	0.42	0.92bc	3.29c	4.14b	3.55bc	3.15c	3.63bc
dried product	0.52bc	0.42	0.94bc	2.94c	3.46b	3.66bc	3.78bc	3.91bc
Storage at 5 degrees (3 days)	0.31c	0.34	0.65c	5.13ab	4.70ab	4.31b	4.16bc	4.24b
p-value	***	Ns	***	****	**	****	****	****

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: ns, **, *** and **** = not significant, significant at $P \leq 0.01$, 0.001 and 0.0001 respectively.

Cluster 3 shows close similarities between aldehyde and dimethyl sulphide during the processing steps, raw materials prior to processing emitted the lowest amounts of aldehydes and dimethyl sulphide, which increased to the highest amount after coring and cutting the iceberg lettuce but reduced during the washing and drying steps; but storage after 3 days caused a rise in the emitted amounts. Detailed description of the volatile changes are below. In relation to the processing steps (rows), Cluster 1: shows close relationship between the quality variables of raw material and the cored and cut product, while Cluster 2: similarities among the quality variables of the 1st washed product, the 2nd Washed product and Dried product as well as the 3 day stored product.

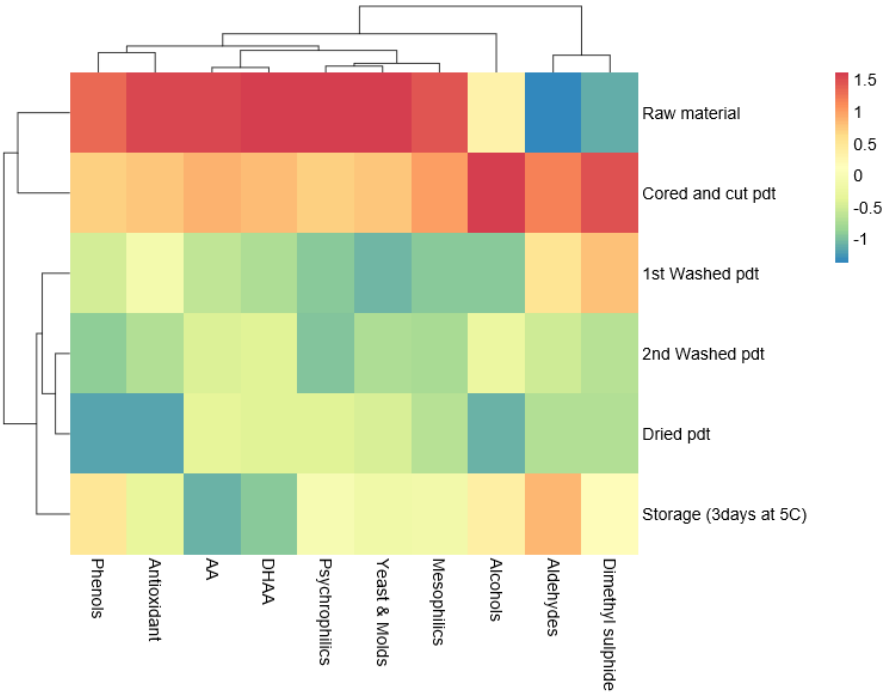


Figure 1.13: Cluster analysis and heat maps of phytochemical, volatile groups and microbial quality of iceberg lettuce as affected by operation mode and processing steps of processor A.

In the heat map of Figure 1.13, volatiles have also been included, since only 3 compound classification with relative area ≥0.01- were found; these were aldehydes, alcohols and Dimethyl sulphide. The other volatile compounds classified were phenols, D-limonene, pyrazines and ketones, but their relative areas were below the 0.01 value. However a total of 21 individual compounds were identified and classified as follows; Aldehyde**s** (7)-Acetaldehyde, Hexanal, 2-Methylpropanal, 2/3-Methylbutanal, Benzaldehyde, Benzene acetaldehyde; Alcohols (7) - Ethanol, 2-Butanol, 1-Hexanol, *(E)*-3-Hexen-1-ol, *(Z)*3-Hexen-1-ol, 2-Ethyl-1-hexanol, 1-Octanol; Ketones (2) – 2-Butanone, 6-Methyl, 5-hepten-2-one; Pyrazines (2) – 2-Methoxy-3-(1-methylpropyl), pyrazine, 2-Methoxy-3-(2-methylpropyl), pyrazine;) Phenols (1); D-limonene and Dimethyl sulphide. All these compounds were also listed by other authors (Deza-Durand and Petersen, 2011; Fischer and Scott, 1997; Palermo, 2012; Lonchamp *et al*., 2009; Palermo, 2012; Tudela *et al*. 2013). .

As for volatiles, Figure 1.13, shows strong relationship between aldehydes and dimethyl sulphide and explains that these two volatiles increased together after the coring and cutting. This occurred probably due to the oxidative effect of the coring and cutting steps. Increased aldehyde volatiles (majority of which was hexanal- a C6 lipid compound) could have been due to the oxidative cleavage of hydroperoxy fatty acids through the action of hydro peroxide in the lipoxygenase-hydro peroxide lyase pathway (El hadi *et al*., 2013). Dimethyl sulphide is an off-odour volatile produced in many vegetables, as a stress indicator Jacobsson *et al*. (2004) and therefore its increase could also be in response to the oxidative stress. This is in accordance with a report by Mastrandrea (2015). Similarly, alcohol levels increased after coring and cutting step. However Figure 1.13, depicts that the washing and drying steps decreased dimethyl sulphide, aldehydes and alcohols that were produced in response to cutting. These results support the finding that aldehydes and alcohols were the major contributors to changes in the aroma characteristics of iceberg lettuce as reported in other studies (Yang, 2008).

Among volatiles aldehydes (mainly acetaldehyde, hexanal and benzene acetaldehyde), increased after storage, whereas a significant increase of ketones (mainly 2-butanone) was observed, even though the value was lower than 0.01, (data not shown). Hexanal (fresh grass, fruit, green and oil flavour) was found in MAP storage and could be associated with rancidity despite their fresh green aroma. They have also been found to be a marker of peroxidation of fatty acids, indicating lipid peroxidation in during the storage period (Shahidi, 2001; Cozzolino *et al*., 2016; Deza-Durand, 2013). Decreased oxygen levels in MAP packages may have led to anaerobic fermentation, resulting in acetaldehyde (floral, ether, pungent and sweet flavour) production which has been found to be a primary cause of off-flavour development (Belay *et al*., 2017). Fermentative odours were found in other minimally processed leaf product in low oxygen MAP storage after 2 days at low temperatures (Rux *et al*., 2017). Benzene acetaldehyde (honey, nut pungent flavour) as detected is a phenylalanine (PAL)-derived product Flament (2002) and may have

increased in storage due PAL activity and phenolic production. 2-Butanone (ketone), which increased in storage at low O_2 (<1%) could have been a product of anaerobic respiration in stored iceberg lettuce; in accordance with studies by Deza-Durand (2013).

D-Limonene, though in trace amounts, significantly decreased due to low oxygen in the packaging environment (data not shown) and this is an indication of limited chlorophyll oxidation and changes in the colour properties of the packaged product in storage. Beaudry (2000) also recounted the inhibition of chlorophyll degradation in low O_2 conditions. Though alcohols, phenols and dimethyl sulphide increased in storage, their concentration were not considerably different from both the raw material and the finished products.

1.5.2.3 Effect of operation mode and processing steps on quality of fresh-cut Romaine Lettuce

In the case of Romaine lettuce minimal processed by processor B, raw material total phenol content and antioxidant activity slightly decreased with chlorogenic acid and p-Coumaric acid during the processing steps, but were not significantly different ($p<0.05$) from the spin dried product (Table 1.19; Figure 1.14).

The minor loss of phenols after cutting may be due to their oxidation (Amodio *et al*., 2014). Noticeable increase in glucose levels from 3.14mg/g d.w to 7.76mg/g d.w after coring and slicing of romaine lettuce at low temperatures could also have been due to phosphorolytic starch degradation (Figure 1.14). Brain Adams (2010) confirmed this in a statement that sugars appear to be formed as a result of phosphorolytic starch degradation in the amyloplast catalyzed by phosphorylases, rather than hydrolytic degradation catalyzed by amylases. However, final sugar concentration after spin drying was lower than that of the initial raw material.

The highest microbial count was observed during the coring and slicing steps but the washing steps was very effective in reducing microbial load (Table 1.19; Figure 1.14). The Cored and sliced products showed, in fact, an increased microbial load of 0.80 log CFU/g for MC, 0.88 log CFU/g for PC and 1.35 log CFU/g Y&M, but after the 2nd wash, there was a significant decrease in microbial counts for all groups analyzed in this study. In comparison to the raw material, the resulting spin dried product had log reductions in microbial count of 1.94, 1.34, 1.33 and for MC, PC and Y&M respectively, equivalent to reductions of 37 %, 25 % and 29 %.

Table 1.19: Effects of processing steps of Processors B on the quality characteristics of Romaine lettuce

	PROCESSOR B					
Processing Steps	**Quality Characteristics**					
	Phenols mg gallic acid/g dry wgt	**Chlorogenic acid mg/g dry wgt**	**Antioxidant mg trolox/g dry wgt**	**MC log CFU/g**	**PC log CFU/g**	**Y&M log CFU/g**
Raw Material	2.80	0.023	2.21	5.30b	5.27b	4.60b
Cored and sliced pdt	2.49	0.011	2.12	6.10a	6.15°	5.95a
1st Washed pdt	2.52	0.006	1.50	5.20b	6.06°	5.26ab
2nd Washed pdt	2.33	0.007	1.65	3.53c	4.11c	3.14c
Spin dried product	1.94	0.008	1.26	3.36c	3.93c	3.27c
p-value	ns	ns	ns	****	****	****

Values are means of triplicate samples. Within each column, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test. Significance: ns, **, ***and **** = not significant, significant at $P \leq 0.01$, 0.001 and 0.0001 respectively

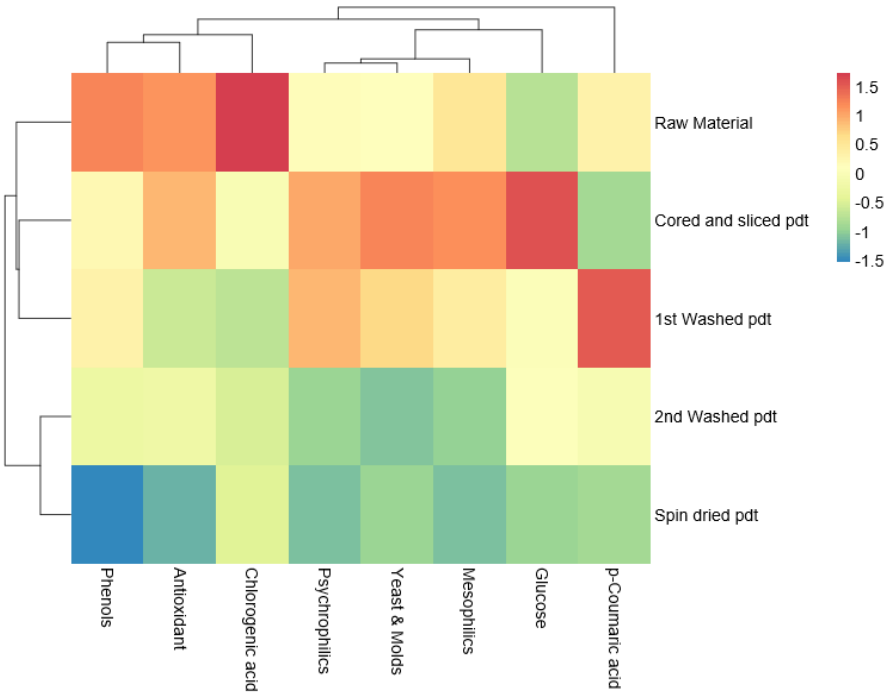


Figure 1.14: Cluster analysis and heat maps of phytochemical and microbial quality of Romaine lettuce by processor B during the main fresh-cut processing steps

Also in this case, a total bacteria screening assay was carried out to confirm the results of bacteria counts (Figure 1.15) using RT-PCR. The results were in line with microbial counts reported in Table 1.19. The recorded increase in sample fluorescence above the established baseline value of 15 (Figure 1.15), is known to be proportional to the amount of the accumulated PCR product during the processing steps. Table 1.20 shows low fluorescence threshold cycle values for raw material rocket leaves which increased during the processing steps, indicating that microbial count decreased during the processing steps.

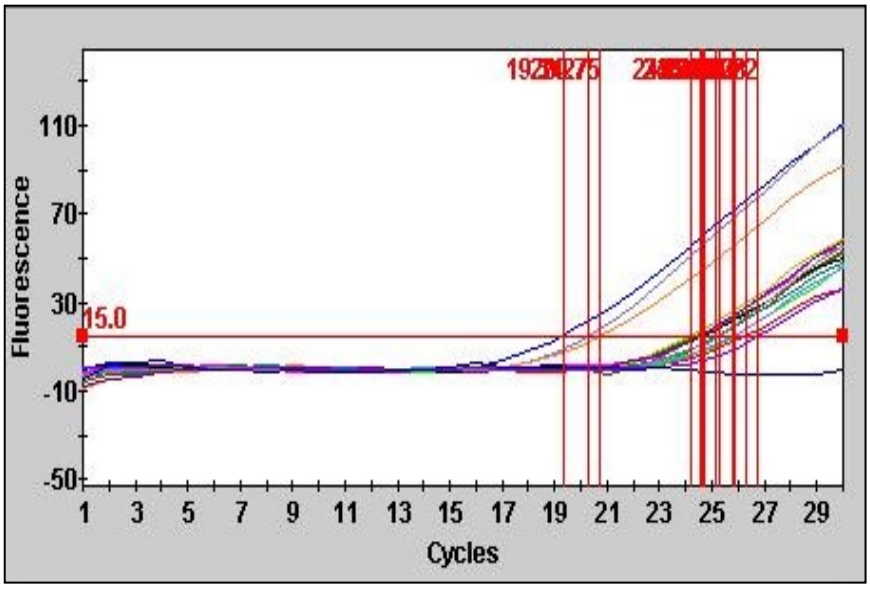


Figure 1.15: Detection cycles of total bacteria during the minimal processing steps of Processor B

Table 1.20: Mean FAM Ct values for Total Bacteria Screening Assay

Processing Steps	RT-PCR Results	
	TBS (FAM)	**FAM Ct***
Raw Material	+	20.12
Cored and sliced pdt	+	24.65
1st Washed pdt	+	25.97
2nd Washed pdt	+	24.87
Spin dried product	+	25.51
Negative control	-	0

*FAM Ct (fluorescence threshold cycle value based on a fluorescein dye)
Low FAM Ct value represents high microbial counts and vice versa

1.6 Conclusion

The most important quality parameters of minimally processed vegetable products are their appearance, microbial quality and phytonutrient components (Rico *et al*., 2007). Minimal processing steps, the mode of operation of different processors, the type of product and storage conditions influenced the final product quality at varying degrees. Although differences were found among processor and raw material quality, generally the cutting and washing steps were the most critical for quality characteristics (volatiles, phytonutrients and microbiology).

Generally a reduction in microbial counts was observed on the final product, but for Processor C, which recorded increase in microbial load probably from cross contamination, which is a risk in minimal processing industry.

Irrespective of sanitizer or mode of processing, an increase of phytochemical metabolism occurred during the washing steps whereas a further oxidation was caused by either spin drying or tunnel drying in rocket leaves, with the result that general nutritional content was not really affected by processing.

As for volatiles, an increase of compounds responsible of off-odors was observed for rocket leaves. Typically, the production of dimethyl sulphide during the minimal

processing operations was observed resulting in poor rocket leaf quality after 2 days of storage for processor A. In addition to ketones which increased in stored rocket leaves from processor A. In processor B, 4-Methylpentyl isothiocyanate decreased during the processing activity with increasing level of off-odor volatiles (Benzaldehyde, Methyl thiocyanate and Dimethyl sulphide) after 5 days of storage.

As for lettuce the cutting stress induced the loss of its phytonutrients, irrespective of the type of lettuce (iceberg and romaine lettuce) and variations in the operation mode of the two processors, though losses in phytonutrients for Romaine lettuce were moderate. For both Iceberg lettuce and Romaine lettuce microbial quality was markedly reduced after processing. Cutting stress on iceberg lettuce induces an increase in stress related volatile compounds (alcohols, aldehyde and dimethyl sulphide) which are minimized by washing steps, though storage for 3 days the stress volatiles to increase in emission.

It can be concluded from this study that the critical step for phytochemical, nutritional, microbial and volatile changes during minimal processing are the cutting and washing steps, as they affect the final product and storage quality. The minimal processing steps and mode of operation by different processors from different locations did not result in a major distinction in minimally processed products with respect to their raw material.

2.0 Effects of air tunnel drying parameters on quality of minimally processed rocket leaves (Diplotaxis tenuifolia) during storage

2.1 Introduction

Rocket leaves have been accepted into the minimally processing and ready to eat food industry for their spicy-pungent and bitter flavour, and are consumed in fresh salads or as a cooked vegetable in many parts of the world (Morales and Janick, 2002) Rocket salads are rich in polyphenols, flavonoids, vitamin C, glucosinolates and other health promoting substances (Bennett *et al*., 2006). However, minimal processing of vegetables may initiate membrane deterioration, inducing water loss and susceptibility to microbial contamination (Artés *et al*. 2007; Gil *et al*. 2009; Escalona *et al*. 2010).

The quality and shelf life of packaged minimally processed leaves like rockets are dependent, among other factors, on the amount of moisture remaining on the surface of the product after washing. Hence, the importance of the drying step, which is usually done, prior to packaging. Unlike the conventional food drying process, wet surface drying in minimal processing requires procedures and technologies that ensures that both visual and internal (chemical composition and bioactive compounds) fresh product characteristics remain intact. As a result, a couple of thermal and non-thermal drying technologies have been developed to efficiently remove surface moisture, prior to packaging. These include the most commonly used centrifugation method, vibration screens or racks, gentle removal with cheesecloth, rotating conveyors, hydro sieves, forced air and spin less drying tunnels (Gorny *et al*. 2002). However, the choice of drying equipment and the extent to which surface moisture is removed during minimal processing is dependent on the type of product. For instance, for baby carrots, excessive moisture removal during centrifugation can cause the development of white blush on product surfaces, while in lettuce and other leafy vegetables, a slight desiccation of the product may provide longer post-processing life (Moretti *et al*. 2007; Cantwell,

2000). Yet excessive centrifugation also causes loss of quality of leafy vegetables through tissue degradation and initiation of biochemical changes through wounding.

Sensory characteristics and microbial quality of minimally processed products influences, consumer purchasing intent and safety. As a result, though some centrifuges have been developed for baby and adult leaves; in order to obtain a final product with minimum or no wounding, good visual quality and extended shelf-life, air drying tunnel is preferred by most industries for minimal processing of rocket leaves (personal communication, 2016). However, like all other drying machines, operating the equipments at the right temperature, speed and time is critical for efficient moisture removal. In addition, operating settings may vary from product to product, due to different morphological, physiological (transpiration and respiration rate) and biochemical properties.

Though air-drying tunnels have been successfully adapted into the minimal processing industry, information on the effects of its drying parameters (temperature, speed and time) on the final product quality is limited. This study evaluated the effects on two drying treatments that ensured residual surface moisture below 2 % on quality and shelf-life or minimally processed rocket leaves.

2.2 Materials and Methods

2.2.1 Materials

Rocket leaves (cultivar: Extrema) from the first cutting of the spring harvest was used for the experiment. Packaging film was polypropylene (PP), with 30 micron thickness (OTR = 1800 $cm^2m^2d^{-1}$ and WVTR = 6 gm^2d^{-1}) with bag dimension of 25 cm x 25.4 cm (height, 25 cm and diameter, 25.4cm). Two air-tunnel drying treatment were tested, one of which was the common company practice.

i) **Treatment A (T-A): Process hot air temperature**: 33 °C, belt length of 7 m and **speed of belt**: 0.022 m/s, with **time** of 5.37 mins. **Air ventilation** was set at 100 %.

ii) **Treatment B (T-B): Process hot air temperature**: 40 ^{o}C, belt length of 7 m and **speed of belt**: 0.026 m/s, with **time** of 4.45 mins. **Air ventilation** was set at 100 %.

The dryer was made of 3 dehumidification units and a one chilling unit, facilitated by a hot block (5 m length) and a cold block (2 m length) respectively. The mechanism for dehumidification was based on hot dry air which dries product by absorbing moisture, after which moisture is removed by ventilation and cooling. After dehumidification, the product enters into a cooling unit, where the product is cooled through ventilation and cooling. The cooled air is then reheated and the process continues in a cyclic order as in Figure 2.1 below. Ambient temperature was 15 ^{o}C. Dewatering speed, belt length, cold air temperature, dew evaporator temperature and aspiration speed remained constant for both treatments.

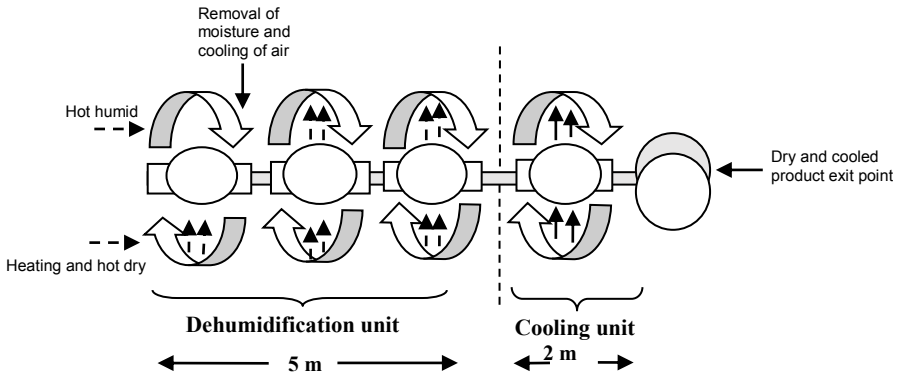


Figure 2.1: A sketch of an air tunnel dryer used for the experiment

2.2.1.1 Sample preparation and handling

The experiment was conducted at a minimal processing company on the 27th of April, 2016, under environmental temperature conditions of 15^{o}C. Initial surface water of 20%, after washing, was reduced to 1.9% and 0.4% for treatment A and B, respectively. 'Surface' dried products of about 100g each were bagged into polypropylene (PP) bags and transported to the laboratory of the postharvest unit,

University of Foggia and stored in a cold chamber at 5ºC. Quality evaluations were conducted on 3 replicates (except where specified) after 5, 9 and 15 days.

2.2.2 Methods

The analysis of the following quality parameters were carried out as described from the previous experiment (see paragraph 1.3);

-Headspace Analysis

-pH, Brix and Titratable acidity,

-Phenol content,

-Antioxidant activity,

-Total Vitamin C,

-Ascorbic acid (AA),

-Dehydroascorbic acid (DHAA)

-Aroma volatiles

-Microbiological analysis

2.2.2.1 Empirical surface water determination

Due to the difficulty of tracking a weighed sample of leaves on the processing line, determination of surface water percentage on the product prior and after drying was empirically calculated as the percentage in weight of the residual water which was possible to absorb with a cheese cloth, over the total wet weight. The amount of water was calculated as the weight gain of the cheese cloth after gentle removal of the water from 100 g of wet leaves sampled after the dewatering obtained with the vibrating conveyor belt transporting the leaves to the drying tunnel.

2.2.2.2 Instrumental Colour

The color was determined using a color reader (CM 2600d, Konica Minolta, Japan) and expressed as CIE L^* a^* b^* units. L^* indicates luminosity or brightness, a^* corresponds to greenness (−)/redness (+), and b^* corresponds to blueness (−)/yellowness (+). The colorimeter was calibrated on the standard tile with a reference value of L*, a* and b* corresponding to 97.76, -0.06 and -0.30

before use. Readings were taken at five points (close to the edges where browning may occur, the central part, the apex and petiole area) on ten leaf samples for each replicate treatment.

2.2.2.3 Total Chlorophyll

One (1) gram of frozen rocket leaf samples were weighed into 25 mL of methanol and kept in the dark for 24 hours at room temperature. 15mL methanol was then added and kept for another 24 hours until no green pigments were found on the leaves. The extracts were then separated and the absorbance was read at resolution of 1 nm using Shimadzu UV-1700 spectrophotometer. Chlorophyll was determined as the maximum absorbance at 66 6nm, 653 nm and 470 nm and the amount of pigments were calculated for chlorophyll a, chlorophyll b and total chlorophyll according to the methods of (Wellburn, 1994).

2.2.2.4 Sensory Evaluations

Organoleptic characteristics of minimally processed rocket leaves were assessed before packaging and during cold storage by an expert panel of 5 people from the Postharvest Unit of University of Foggia. After opening the bags, off-odor, visual quality, texture and aroma were evaluated in sequence, using descriptive scales from 5 to 1.At the end of the test, off-odors evaluation was assessed again to evaluate its persistence after broking the leaves. For the off-odors scale, 1 represented no off-odors and 5 represented very strong off-odors /sulfur compounds. For **Visual quality** the scale included the following points; 5=fresh and turgid appearance, bright and uniform green color; 4=slight loss of turgidity and fresh appearance; 3=moderate/noticeable loss of turgidity (limit of marketability); 2=severe loss of turgidity, wrinkling and yellowing of leaves (limit of edibility).; 1=severe yellowing of leaf blades, wilting or possible decay For **Texture** the evaluation was based on finger feel sensation; 5=excellent, fresh and succulent; 4=very good, moderately firm; 3=good, slightly firm; 2=fair, moderate softening; 1=poor, limp and wilted. **Aroma** was scored as following; 5= typical/strong (already perceptible on intact leaves); 3= typical (perceptible on broken leaves); 1=

slight (the odour perception was limited to rubbed and manipulated leaves). Purchase intent (value for money) of panelists were subsequently determined using the following scoring scale where 5= definitely would buy; 4= probably would buy; 3=indifferent; 2=unlikely to buy; 1= definitely would not buy).

2.3 Statistical analysis

Two way-anova analysis was carried to assess the effect of drying treatment and storage time, using STATGRAPHICS Centurion software (XVI.I version, Stat point Technologies, Inc., 2009). Mean separation was achieved by applying Tukey's honest significance difference test ($p<0.05$). A Principal Component Analysis (PCA) was applied to visualise the variation in aroma volatile composition during the storage period using Statistica software (ver.7, Stat Soft, Tulsa, OK, USA)

2.4 Results and discussion

2.4.1 Quality of raw material and resulting minimally processed rocket leaves after tunnel drying

Raw material quality is critical in the minimal processing industry as it influences the resultant final product quality, irrespective of processing treatments. Initial raw material prior to minimal processing and drying treatment was scored 4.8 ± 0.3 for visual quality, 4.5 ± 0.5 for texture and 5 for purchase intent; only aroma received a score of 3.0 ± 0.3, meaning it was only perceptible when leaves were broken (Table 2.1). No off-odors were perceived. For microbial quality, mesophilic and psychrophilic counts were below the limit for contamination (8 log CFU/g) while yeast and molds reached already the limit for spoilage fixed in 5 log CFU/g as suggested by Jacxsens *et al*. (1999). The raw material quality properties as shown in Table 2.1, slightly changed after minimal processing and drying (Table 2.2). Sensory quality of the dried products of both T-A and T-B were mostly the same and were not significantly different from the raw material prior to processing, however in the case of microbial quality, though changes were not significant between T-A and T-B samples, microbial loads had reduced by 0.94 and 0.77 log CFU/g (for psychrophilic bacteria), 0.67 and 0.73 log CFU/g (for yeast and molds),

0.70 and 0.41 log CFU/g (for mesophilic bacteria) for T-A and T-B samples respectively, compared to the raw material. Reduction of microbial levels of the raw material could be due to the washing and sanitizing steps during minimal processing of the raw material rather than to the tunnel drying. The effect of washing and sanitization on the reduction of microbial load of leafy vegetables during minimal processing has been reported by several authors (Nascimento *et al*., 2003; Singh *et al*., 2002; Martínez-Sánchez *et al*., 2006).

Table 2.1: Sensory and microbial quality of raw material used for the experiment

Raw material	Sensory Quality Score				Microbial Quality (Log CFU/g)		
	Visual Quality	Texture	Aroma	Purchase intent	Mesophilic count	Psychrophilic count	Yeast and molds count
Rocket leaves	4.8 ± 0.3	4.5 ± 0.5	3.0 ± 0.3	5.0 ± 0.0	6.9 ± 0.4	4.1 ± 0.3	5.0 ± 0.0

Rocket leaves sorted but prior to minimal processing and drying were used for the above assessment and analysis respectively. Off-odor initial and Off-odor persistent were relevant only for stored samples and hence are omitted from the above table. Values are means ± standard error

Table 2.2: Sensory and microbial quality of tunnel dried rocket leaves prior to storage

Inital quality (dried product)	Sensory Quality Score				Microbial Quality (Log CFU/g)		
	Visual Quality	Texture	Aroma	Purchase intent	Mesophilic counts	Psychrophilic counts	Yeast and molds counts
T-A	4.5 ± 0.0	4.2 ± 0.2	3 ± 0.3	4.5 ± 0.3	6.2 ± 0.2	3.2 ± 0.5	4.4 ± 0.0
T-B	4.5 ± 0.0	4.5 ± 0.0	3 ± 0.0	4.5 ± 0.3	6.5 ± 0.0	3.4 ± 0.0	4.3 ± 0.1

Rocket leaves minimal processed and dried following treatments A (T-A) and treatment B (T-B) were used for the above assessment and analysis respectively. Off-odor initial and Off-odor persistent were relevant only for stored samples and hence are omitted from the above table. Values are means ± standard error

2.4.2 Changes in headspace gas concentration over storage time

The concentration of oxygen (O_2) decreased with increasing carbon dioxide (CO_2) in the packaging of leaves from both T-A and T-B during the storage period, reaching an equilibrium at the 13th day of storage at 5^{o}C (Figure 2.2). Similar phenomenon has been reported (Koukounaras *et al*., 2007; Spadofora, 2017) during storage of rocket leaves. No differences in gas were observed between treatments; O_2 levels reduced to 11.73% and 11.53% while Carbon dioxide (CO_2) increased to 7.53% and 7.50% after 15 days of storage at 5^{o}C (Figure 2.2). Maintaining aerobic conditions for storage quality of rocket leaves has been recommended by Luca *et al*. (2016). This was achieved throughout the storage period for both treatment samples in this study.

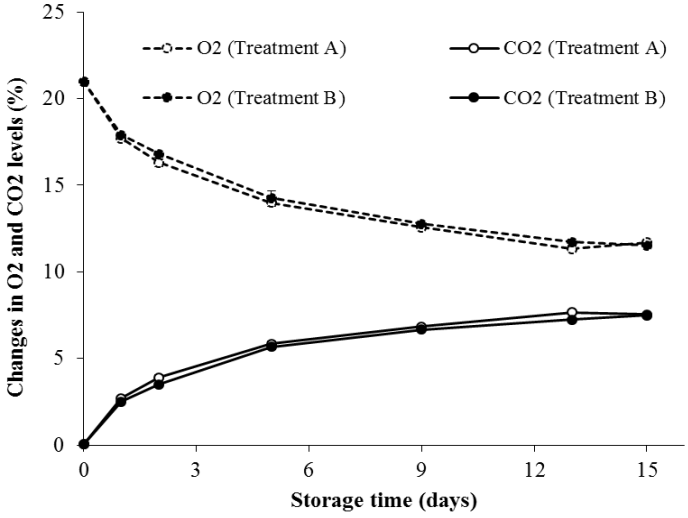


Figure 2.2: Oxygen and carbon dioxide changes over storage at 5 °C of rocket leaves subjected to drying treatment A and treatment B.

2.4.3 Changes in Colour, pH, Total soluble solids (TSS) and Titratable acidity in response to drying treatment and storage time

The effect on drying treatment and storage time on colour and other quality attributes is shown in Table 2.3. The L*, b* and chroma angle showed significant effects ($p \leq 0.05$) of the drying treatment on the colour of rockets leaves. Rocket leaves dried with treatment A, had higher L*, b* and chroma values indicating

lightness and slight yellowness (data not shown) than rocket leaves dried with treatment B. The colour difference may have also been due to the presence of water on leaf surfaces causing an alteration in the reflective properties of the light (Heusinkveld *et al*., 2008). The a* value which was negative, did not change significantly, confirming that almost similar green component of rocket leaves was observed for both treatments treatment A (ranging from -7.9 ± 0.25 to -7.7±0.21) and treatment B remained stable at -7.3. Similarity in the greenness value after drying and storage under different conditions have also been reported in other brassica leafy vegetables like kale (Araújo *et al*. 2017). However, storage time significantly influenced ($p \leq 0.0001$) overall changes in colour (ΔE), such that despite similar changes in colour parameters (Table 2.3) during storage, overall colour changes for packaged rocket leaves dried with treatment A (2.92) were higher than that of treatment B (2.87) after 15 days of storage at 5°C. However, the colour difference was less 6. Colour difference of less than 6 has been reported to be imperceptible to the human eye.

Changes in pH, total soluble solids and titratable acidity were mainly influenced by storage duration, nonetheless, variation in titratable acidity of the rocket leaves was also influenced by drying as well as by its interaction with storage time (Table 2.3). Significant increase in titratable acidity (% citric acid) was observed after 5 days of storage, especially for treatment A, from 0.28 ± 0.02 % to 0.47 ± 0.02 %, with decreasing pH from 7.4±0.03 to 6.9±0.02, compared to treatment B (stable at - 0.28 ± 0.03%) with decreasing pH from 7.3±0.0 to 7.1±0.1. Similar results have been observed in MAP storage of fresh-cut fruits and vegetables at 5 ^{o}C resulting from the growth of lactic acid bacteria (Aguayo *et al*., 2014; Nguyen-the and Carlin 1994). Also TSS changed over storage time, but with a slowest rate, showing an increase from 6.6 ± 0.2 to 8.0 ± 0.0 for treatment A and from 7.5 ± 0.1 to 8.2 ± 0.1 for treatment B after 15 day storage and may be due to MAP storage conditions (Sharma *et al*., 2012; Pereira *et al*., 2014); on reducing the rate of loss.

Table 2.3. Effect of drying treatment, storage time and their interaction, as resulted by a 2-way ANOVA, on quality characteristics of rocket leaves subjected to drying treatment A and B during 15 days storage in MAP at 5 °C .

Quality characteristic	Treatment A	Treatment B	Drying treatment	Storage time	Drying treatment x Storage time
L*	46.65 a	45.85 b	*	ns	Ns
a*	-7.57	-7.23	ns	ns	Ns
b*	16.52a	15.53b	*	ns	Ns
Chroma angle	18.17a	17.12b	*	ns	Ns
Hue angle	114.72	114.81	ns	ns	Ns
ΔE	2.92	2.87	ns	****	Ns
pH	7.08	7.09	ns	****	*
TSS	7.56	7.73	ns	**	Ns
%TA	0.34a	0.28b	***	***	**

Significance: ns, *, **, *** and **** = not significant, significant at P ≤ 0.05, 0.01, 0.001 and 0.0001, respectively

2.4.4 Effect of drying treatment and storage time on microbial and sensorial quality

Figure 2.3 and two-way anova results (data not shown), shows that changes in microbial quality of rocket leaves was not significantly influenced by the drying treatment but rather by the storage duration with the exception of psychrophilic bacteria which showed significant differences between treatment A and B particularly after 15 days of storage as well as the interaction between drying treatment and storage time ($p \leq 0.05$). Growth of micro-organisms were delayed by the modified atmosphere storage, such that mesophilic and psychrophilic bacteria counts were within the limit of 8 log CFU/g proposed by Debevere (1996). Yeast and molds on the other hand, exceeded the limit of 5 log CFU/g (above which spoilage can be detected by consumers) after 9 days of storage (Debevere, 1996 and Jacxsens *et al*., 1999) as shown in Figure 2.3, but it should considered that 9 days had already exceeded the commercial shelf-life normally fixed at 6 -7 days in Italy (Lunati, 2003). Passive atmosphere storage did not halt the growth of microorganisms, but most likely it contributes to some delay; microbial

proliferation during storage of minimally processed products is more dependent on temperature than modified atmosphere (Zagory, 1999; Jacxsens *et al*., 2002).

Though the initial psychrophilic bacteria load was comparatively lower than mesophilic group, the growth of this bacteria was faster during storage at 5 ^{o}C (Figure 2.3 A). This is because, as expected, the cold storage temperature favoured the growth of psychrophilic bacteria and decreased the growth mesophilic bacteria (Nguyen-the and Carlin, 1994). Significant rise in psychrophilic bacteria in samples from treatment A may be attributable to higher residual surface moisture of 1.9 % (close to the 2 % limit) compared to 0.4 % observed in samples from treatment B. Free or residual surface water contributes to decay and microbial spoilage in minimally processed products (Bolin *et al*., 1997; Barth *et al*., 2009). Despite the less reduced residual moisture of treatment B, drying efficacy may be improved as drying time is reduced by approximately 1 minute (0.92 mins).

Also, sensory quality properties were decreasing mainly due to the storage time, $p \leq 0.05$, and showed some differences in texture and aroma at day 5, with leaves from treatment A showing higher score values than for B (Figure 2.4). Significant negative correlation was found between visual quality and psychrophilic bacteria ($r = -0.74$ at $p \leq 0.0001$), and yeast and mold counts ($r = -0.72$ at $p \leq 0.001$) during the 15 days of passive MAP storage at 5 ^{o}C (data not shown). This indicates that the decrease in visual quality from 4.5 (at day 0) to 3 (at day 15) for both treatments (Figure 2.4) was also influenced by the increasing microbial contamination during the storage period. Psychrophilic bacteria (for example pseudomonas sp.) and yeasts and molds have been reported to have pectinolytic and cellulase enzyme activity on stored minimal processed products (Jacxsens *et al*., 2002; Babic *et al*., 1992; Laurent *et al*., 2000). Visual defects in minimally processed products resulting from micro-organisms are often associated with texture breakdown and quality differentiation at purchase (Ragaert *et al*., 2007). This could explain the decrease in visual quality, texture quality, and purchase intent with the increase of the psychrophilic bacteria counts (Figure 2.3 and Figure 2.4), However sensory

quality properties did not decreased below the limit for acceptability (3) throughout the storage period of 15 days. With regards to off-odors, apart from the initial off-odor, which increased (to 2) after 5 days of storage probably due to MAP conditions, no significant persistent off-odor was detected during the storage period, probably because, off-odor development is associated with decay and tissue softening (Medina *et al*., 2012) and this was not the case of this study. The maintenance of quality of rocket leaves during the storage period could be due to passive modified atmosphere reducing physiological activity and its corresponding microbial contamination.

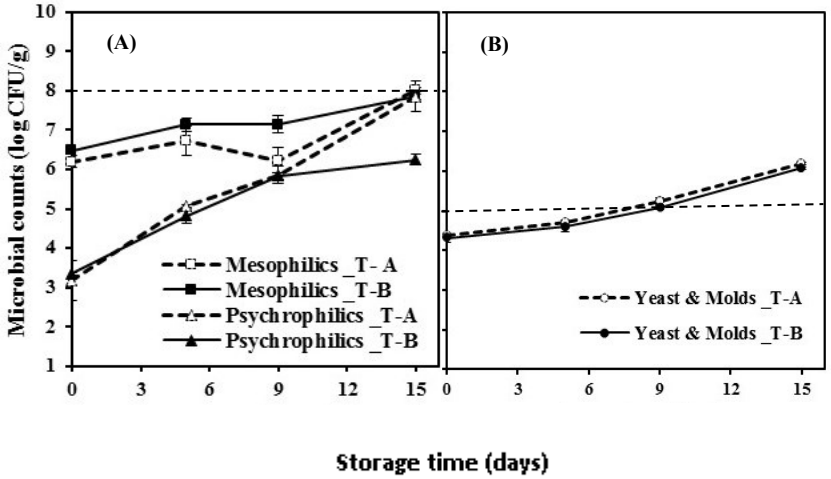


Figure 2.3: Mesophilic bacteria and psychrophilic bacteria (**A**) and Yeast and mold counts (**B**) on minimally processed rocket leaves subjected to drying treatment A and B during 15 days storage in MAP at 5 °C. Error bars represents standard error of triplicate sample means (n=3). The line (- - -) indicates the microbial limits.

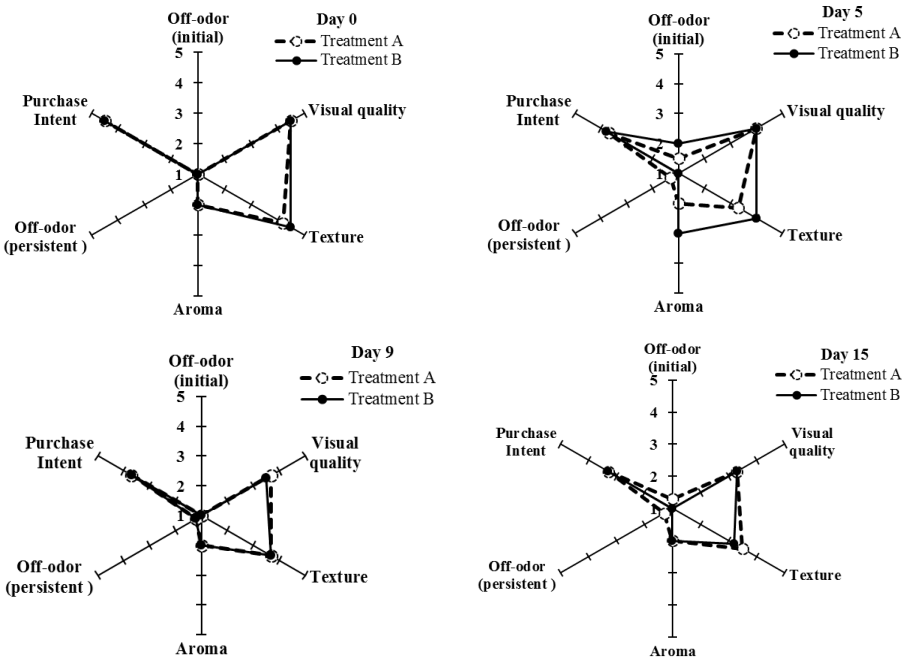


Figure 2.4: Changes in sensorial properties of rocket leaves subjected to drying treatment A and B at day 0, and after 5, 9 and 15 days of storage in MAP at 5 ºC. The scores as shown are based on a 5-point scale system were (5= best; 3=acceptable; 1=worst), except for off-odors (1=least and 5 =strong).

2.4.5 Effect of drying treatment and storage time on phytonutrient content

Drying processes can lead to degradation of naturally heat-sensitive phytonutrients like vitamins, antioxidants, pigments and other bio-active substances (Araújo *et al*. 2017). Moreover, this is dependent on temperature and the exposure times. As observed in this study, although the temperature of treatment A (33 ºC) was lower than that of treatment B (40 ºC), the drying time may have influenced changes in the phytonutritional properties, such that higher drying temperature of B with shorter drying time of 4.45mins helped to retain more phytonutrients than treatment A with lower drying temperature and longer drying time of 5.37mins (Table 2.4; Figure 2.5). Better retention of nutritional properties of leafy greens at a faster drying rate has also been reported by other authors (Negi and Roy, 2001)., Significant differences were found on initial ascorbic acid, dehydroascorbic acid,

total vitamin C and total phenolic content with respect to drying treatment, but for antioxidant activity and chlorophyll content which were not substantially affected (Figure 2.5). Storage time had a significant effect on all phytonutrients analyzed but generally their interaction with drying treatment was not statistically significant.

Noticeable increase ($p \leq 0.05$) in phytonutrient content of rocket leaves after drying was observed after 5 days of storage and may be related to changes in gas composition due to processing stress but also as a consequence of the drying stress, since differences were observed between the two treatments. In the case of treatment A, a rise of 11.90%, 48.30%, 15.13%, 13.38%, 21.19%, 6.89% was observed for ascorbic acid, dehydroascorbic acid, total vitamin C content, total phenolic content, total antioxidant and total chlorophyll content. For treatment B, an increase of 8.99%, 29.09%, 10.35%, 18.19%, 29.85% and 10.78% was observed at the same time of storage. Generally after 9 and 15 days, the same phytonutrient content was maintained, but for dehydroascorbic acid which continued to rise during the storage period (Figure 2.5) and doubling at the end of the storage time for both drying treatments, with higher levels in samples from treatment A than from B. Increase in dehydroascorbic acid during storage has been reported in fruits and vegetables as result of AA oxidation (Mazurek and Pankiewicz, 2012).

Table 2.4. Effect of drying treatment, storage time and their interaction, as resulted by a 2-way ANOVA, on phytonutrients of rocket leaves subjected to drying treatment A and B during 15 days storage in MAP at 5 °C

Quality characteristic	Drying treatment	Storage time	Drying treatment x Storage time
Ascorbic acid (mg/100g fw)	**	**	ns
Dehydroascorbic acid (mg/100g fw)	**	****	*
Total Vitamin C (mg/100g fw)	**	**	ns
Total phenol (mg gallic acid/100g fw	**	****	ns
Total antioxidants (mg trolox/100g fw)	ns	****	ns
Total chlorophyll (mg/100g fw)	ns	*	ns

Significance: ns, *, **, *** and **** = not significant, significant at $P \leq 0.05$, 0.01, 0.001 and 0.0001, respectively

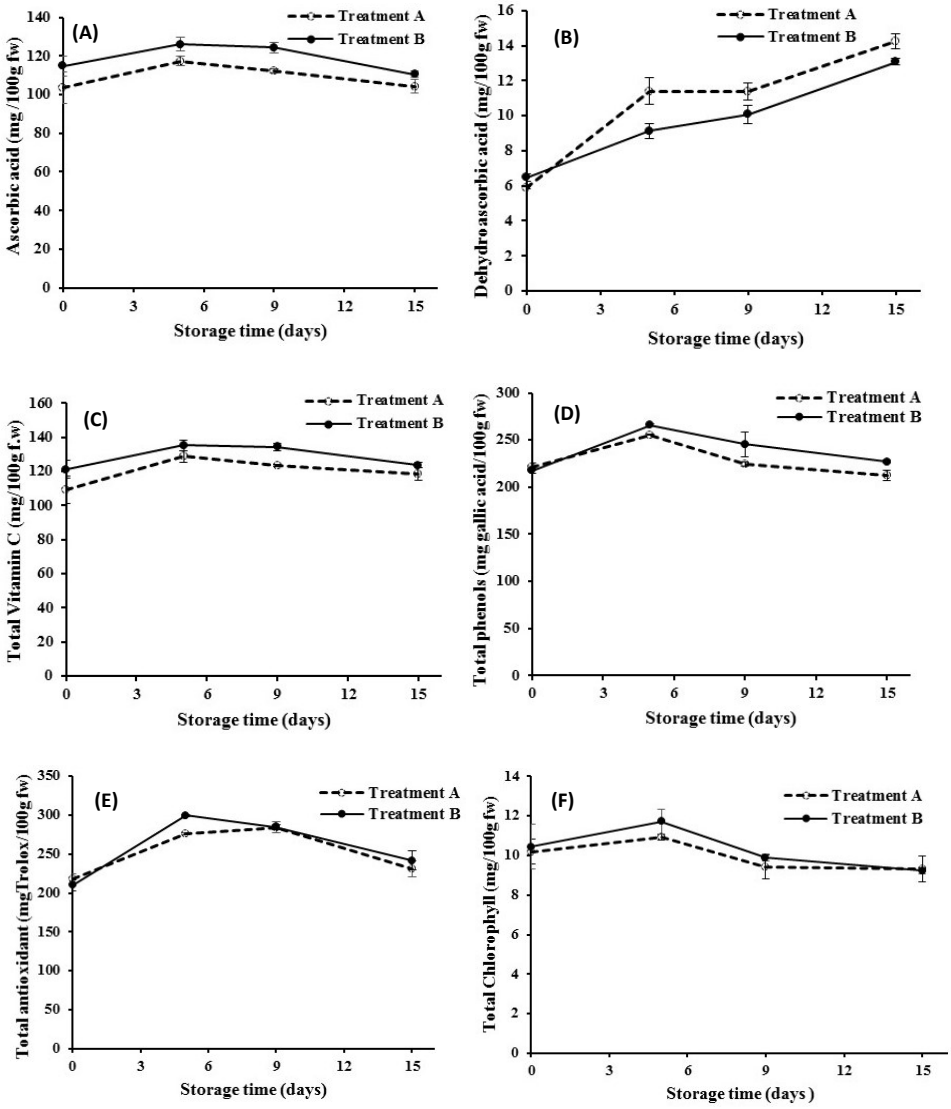


Figure 2.5: Changes in phytonutrient content (A) Ascorbic acid; (B) Dehydroascorbic acid; (C) Vitamin C ; (D) Total phenolics; (E) Total antioxidant activity and (F) Total carotenoids of rocket leaves subjected to drying treatment A and B during 15 days storage in MAP at 5 °C . Error bars represents standard error of triplicate sample means (n=3).

2.4.6 Effect of drying treatment and storage time on aroma volatiles

The volatile compounds detected in drying samples during storage in passive MAP at 5 ^{o}C and their respective descriptors are shown in (Table 2.5). A total of 16 major polar volatiles in grinded rocket leave tissue were identified. Aroma profile of rocket leaves from both grinded and intact leaf tissues have been reported to show no qualitative difference, though higher odor factor is found in grinded tissue paste (Jirovetz *et al*., 2002). All the volatile compounds identified have been characterized in vegetables belonging to the Brassicacea family (Taveira *et al*., 2009), including rocket leaves. The changes in volatile compounds were mainly influenced by the storage time ($p \leq 0.05$), but not by the drying treatment, nor by the interaction between the drying treatment and storage time. The only exception was 2-Ethyl furan which appeared to be significantly higher in rocket leaves from treatment A than from treatment B, without any change during the storage period ($p \leq 0.05$). The evolution of 2-ethyl furan is associated with membrane degradation and has been reported as a possible marker of fatty acid oxidation in wild rocket leaves (Luca *et al*., 2017). This could also explain lower Vitamin C content in rocket leaves from treatment A compared to that of B (Figure 2.5 c), since Vitamin C has been described as an efficient precursor for furan production (Mark *et al*., 2006; Fan *et al*., 2008; Limacher *et al*., 2007; Owczarek-Fendor *et al*., 2012).

Table 2.6, account only for volatile compounds which changed during the storage time, since beside the initial difference between the two drying parameters, no difference due to the drying treatment were observed during storage duration. For this reason, only the compounds showing significant changes over time were included in the principal component analysis. Table 2.6 shows the effect of time of storage on volatile composition over time.

The volatile compounds were summed into their respective belonging groups for the analysis, which were aldehydes, alcohols, ester and ketones, whereas sulphur compounds were analysed individually due the comparatively high significance level ($p \leq 0.0001$) of dimethyl sulphide during the storage period (Table 2.5), in comparison to carbon disulphide and other volatile groups.

The PCA showed that 79.72% of the variance could be explained by two main components having eigenvalues greater than 1. Figure 2.6 shows the score plot (A) and loading plot (B) of the variables in the PCA projected on the plane of first principal component PC1 vs the second principal component PC2, accounting for the variation observed. The score plot (Figure 2.6 A) depicts changes in the volatile properties of leaves from treatment A (T-A) and B (T-B) during the storage period (Figure 2.6 B).

Volatiles are released in green leafy vegetables in response to the storage time, temperature, microbial load and gas composition in packages (Cazzolino *et al*., 2012; Nielsen *et al*., 2008; Luca *et al*., 2016; Amaro *et al*., 2012). At day 0 of storage T-B_d0 and T-A _d0 were positively correlated with high alcohol production and low levels of all the other volatile compounds (ketones, aldehydes, ester, carbon disulphide, dimethyl sulphide, n-Pentyl isothiocyanate). After 5 days of storage (T-A_d5 and -T-B_d5), ester (Z)-3-Hexen-1-ol, acetate), ketones, n-Pentyl isothiocyanate, carbon disulphide, dimethyl sulphide and aldehydes, began to increase (PC1) together with increasing alcohol concentration (PC2). Increase in (Z)-3-Hexen-1-ol acetate could be due to increased yeast and mold counts after 5 days (Figure 2.6 B) as it is produced in response to fungal contamination (Shiojiri *et al*., 2006). All the compounds as mentioned, continued to increase together also after 9 days of storage (T-A_d9 and T-B_d9), until 15 days (T-A_d15 and T-B_d15) where dimethyl sulphide was the predominant compound (PC2). The increasing concentration of dimethyl sulphide during the cold storage period could be a result of accelerated metabolism in response to microbial growth and enzyme activity (Luca *et al*., 2016; Peng *et al*., 2014). Dimethyl sulphide is an off-odor compound which can be produced by microorganisms using sulphurous glucosinolates for their growth (Nielsen *et al*., 2008).

Microbial growth and enzyme activity during the storage period has been related previously in this study. Decreased concentration of aldehydes and alcohols after 15

days together with almost stable concentrations of esters (Table 2.6), can be explained by their conversion to esters (Pelayo *et al*., 2003; Caleb *et al*., 2013).

Table 2.5: Effect of storage time on volatile compounds of rocket leaves subjected to drying treatment A and B during 15 days storage in MAP at 5 °C

Aroma Volatile Classification	RT (min)	Storage time	Aroma/Odor descriptors[a]
Aldehydes			
Hexanal	14.43	**	Fresh, grass, green, oil
(E)-2-Pentenal	16.65	**	Floral, green
(E)-2-Hexenal	20.86	**	Fat, floral, green grass, pungent
(E, E)-2,4-Heptadienal	33.45	ns	Fat, nut, flower, plastic
Benzaldehyde	34.87	ns	Bitter almond, malt, roasted pepper
Alcohols			
1-Penten-3-ol[b]	18.81	*	Pungent, green
(Z)-2-Penten-1-ol	25.63	*	Green, plastic
(Z)-3-Hexen-1-ol	28.55	ns	Green leaf, grass, herb
Furans			
2-Ethyl furan	9.35	ns	Butter, caramel
Esters			
(Z)-3-Hexen-1-ol, acetate	25.42	**	Fresh, leafy, floral, green vegetable
Ketones			
3-Pentanone	7.76	***	Ether, fragrant, pleasant, sweet
1-Penten-3-one	11.85	**	Green, herb, metal, mustard, pungent
Sulphur compounds			
Carbon disulphide	4.92	**	Vegetable sulphide
Dimethyl sulphide	5.12	****	Cabbage, organic sulfur, wet earth
Isothiocyanates			
n-Pentyl isothiocyanate[b]	33.12	*	Green
4-Methylpentyl isothiocyanate[b]	35.34	ns	Pungent, horseradish

All volatile compounds were identified by comparing MS data to spectra from NIST library and previous work in our laboratory (Mastrandrea, 2015)
RT (min): Retention time in minutes; Significance: ns, *, **, *** and **** = not significant, significant at $P \leq 0.05$, 0.01, 0.001 and 0.0001respectively
[a]Odor descriptors were sourced from www.vcf-online.nl_EU-Flavislist
[b]Odor descriptors from published data Jirovetz *et al*. 2002; Sigma-Aldrich, 2000; referenced in Mastrandrea, 2015)

Table 2.6: Effect of passive MAP storage on changes in volatile composition of rocket leaves subjected to drying treatment A and B

Aroma Volatile	**Days of storage at 5 °C**			
Classification	**0**	**5**	**9**	**15**
Aldehydes				
Hexanal	0.056±0.006b	0.080±0.008b	0.081±0.011b	0.009±0.002°
(E)-2-Pentenal	0.009±0.0007b	0.008±0.0009bc	0.014±0.0012a	0.005±0.0002c
(E)-2-Hexenal	0.770±0.062b	0.987±0.069b	1.531±0.222a	0.554±0.037b
Alcohols				
(Z)-2-Penten-1-ol	0.001±0.0005b	0.002±0.0004ab	0.003±0.0004a	0.001±0.0003b
Esters				
(Z)-3-Hexen-1-ol, acetate	0.003±0.001b	0.016±0.001a	0.017±0.001a	0.017±0.002°
Ketones				
3-Pentanone	0.001±0.0007b	0.006±0.0004a	0.006±0.0006a	0.006±0.0005a
1-Penten-3-one	0.018±0.003bc	0.025±0.002b	0.038±0.003a	0.014±0.001c
Sulphur compounds				
Carbon disulphide	0.013±0.001c	0.022±0.0006a	0.019±0.0004b	0.013±0.0005c
Dimethyl sulphide	0.000±0.000c	0.001±0.0002b	0.002±0.0001a	0.002±0.0001a
Isothiocyanates				
n-Pentyl isothiocyanate[b]	0.009±0.003c	0.033±0.002a	0.026±0.002ab	0.018±0.001b

Values are means of 3 replicates. Within each row, values followed by different letters are statistically different ($P < 0.05$), according to Tukey (HSD) test.

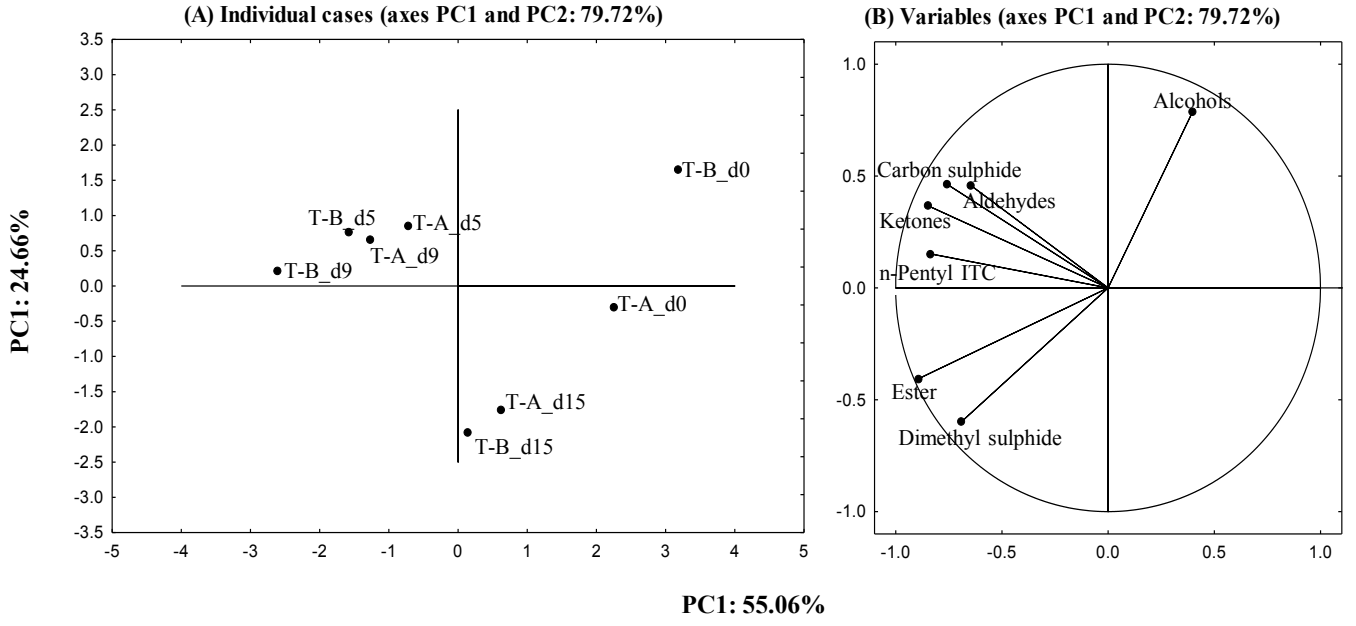


Figure 2.6: PCA analysis on the volatile compounds of the rocket leaves subjected to drying treatment A (T-A) and B (T-B) during 15 days storage in MAP at 5 °C Values are means of triplicates (d0 – day 0; d5 - day 5; d9 – day 9; d15 – day 15; the numbers refer to the days of storage). n-Pentyl ITC (n-Pentyl isothiocyanate)

2.5 Conclusion

The variation of air drying parameters for treatments A and B was generally not inducing significant difference in quality of the final product over storage. Microbial proliferation did not cause significant spoilage as also observed for sensorial quality, since MAP packaging and cold storage temperature help to contain quality changes over time. Slight higher water surface of rocket leaves from treatment A may have affected microbial and phytochemical quality changes of stored rocket leaf products, besides hastening tissue disintegration as seen with higher 2-Ethyl furan emitted by leaf tissue. Processing parameters used for treatment B has potential to reduce drying time and improve storage quality of minimally processed rocket leaves due to resulting low residual surface moisture of products. Regulation of drying time to meet temperature and speed requirements of products, while maintaining optimum ventilation could improve drying, quality and shelf-life of minimally processed rocket leaves and possibly other leafy vegetables.

PART THREE: CONCLUSIONS

3.0 General Conclusions

Minimal processing industry is challenged by physical damages, physiological and biochemical changes influencing quality of raw material and the minimal processed product. Factors like harvesting systems, time of harvest, maturity stage, postharvest handling and storage conditons of fresh produce intended for fresh-cut processing have been reviewed as important contributors to quality of the final product. Much more, the processing steps can greatlyaffect the quality of fresh-cut of leafy vegetables. Tissue damage caused by processing operations induces changes in appearance, phytochemical, microbial and aroma quality properties of fresh-cut products. However the extent of the damage may vary with different type of equipments and different operation modes (cutting equipment, washing conditions, conveyor belt speed, drying temperature and time).

In this study it was found that minimal processing steps, the mode of operation of different processors, the type of product and storage conditions influenced the final product quality at varying degrees. In general, the cutting and washing steps were the most critical points that affected changes in quality characteristics (volatiles, phytonutrients and microbiology).

The processing steps used by the processors sampled in this study reduced microbial counts on rocket leaves and cut lettuce but in same cases (processor C) din not prevent cross contamination. This potential risk factor, indicates that raw material microbial quality from batch to batch during minimal processing should be carefully monitored and that also the plant sanitization deserve more attention.

Metabolism of phytonutrients and particularly accumulation of phenolics and increase in antioxidant activity was observed during the washing steps of rocket leaves processed without cutting, irrespective of processing mode and sanitizers used, as consequence of the washing stress. Generally this increase was not affecting the overall nutrient content of the final product since a further oxidation

was then induced by the spin or tunnel drying. As for lettuce a loss of phytonutrients was observed as consequence of the oxidation induced by cutting, irrespective of the type of lettuce (iceberg and romaine lettuce) and variation in operation modes of the processors.
As for aroma quality, the main changes occurring for rocket leaves were due to the time of storage with the increase of off odor volatiles as dimethyl sulphide and ketones. In other case, a decrease of 4-Methylpentyl isothiocyanate during the processing activity was also observed, with increasing level of off-odor volatiles (Benzaldehyde, Methyl thiocianate and dimetyl sulfide). As for fresh-cut iceberg lettuce, the cutting step and storage period were identified as the critical points for off-odor volatile production (alcohols, aldehyde and dimethyl sulphide).

Furthermore the study of drying conditions on rocket leaf quality, showed that reducing the temperature from 40 to 33 °C and regulating the drying time in order to contain the residual water surface below 2% did not cause considerable effect on phytonutrients and shelf-life.. Passive MAP packaging and cold storage temperature minimized quality changes over time. Slight higher water surface of rocket leaves from the treatment at the lowest temperature, may have affected microbial and tissue disintegration in storage observed by 2-Ethyl furan emitted by leaf tissue, but without inducing sensible variation in sensorial quality. These results suggested that the treatment at 40 °C and shortest time should be preferred in order to increase process productivity, allowing to maintain lower residual water without inducing any thermal degradation.

All these finding contributed to fill the existing gap between physiology of cut tissues and general knowledge about the impact of processing operations on quality of the final product, by increasing the amount and detail of information. The innovative approach of the research was due to the novelty of sampling 2 product models, one whole leaf and one cut, among the most important on the market, from different processors in 2 continents, and analyzing at the same time the impact of the processing steps on several

physical, sensorial, microbial and nutritional parameters, including volatiles. All these aspects would allow processors to improve the management of the critical processing steps and maintain initial raw material quality throughout the productive chain up to the consumer.

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APPENDIX I. SENSORY SCALE FOR ROCKET LEAVES

Rating Scale Rocket (*Diplotaxis tenuifolia*)

Score 5 - Excellent
Fresh and turgid appearance, bright and uniform green color.

Score 4 - Good
Slight loss of turgidity and fresh appearance.

Score 3 - Fair
Noticeable loss of turgidity and possible slight loss of green color.

Limit of marketability.

Score 2 - Poor
Severe loss of turgidity. Wrinkling and yellowing of leaf blades.

Limit of edibility.

Score 1 - Very Poor
Severe yellowing of leaf blades and wilting. Possible appearance of decay.

APPENDIX II
POSTHARVEST UNIT- DEPT. OF S.A.F.E, UNIVERSITY OF FOGGIA
SENSORY EVALUATION ON ROCKET SALAD

PANELIST NAME: ……..
DATE: ……...
INSTRUCTIONS: Read carefully before you start
You have been provided with 6 coded samples. Kindly rate them using the scale below and provide your scores in the table accordingly;

Table of Evaluation for samples provided

Sample code	Off-odor (initial)	Visual quality	Texture (Firmness)	Aroma /odor	Off-odor (persistent)	Purchase Intent

ATTRIBUTE DESCRIPTION SCALE:
Off-Odor (initial):
5= very strong off-odors/sulfur compounds
4= strong off-odors
3= moderate off-odors (limit of acceptability)
2= slightly off-odors
1= no off-odors

Visual Quality:
5= fresh and turgid appearance, bright and uniform green color
4= slight loss of turgidity and fresh appearance
3= moderate/noticeable loss of turgidity, slight loss of green color (limit of marketability)
2= severe of turgidity, wrinkling and yellowing (limit of edibility)
1= severe yellowing of leaf blades and wilting /possible decay

Texture (characteristic firmness); finger feel
5= excellent/fresh/succulent
4= very good; moderately firm
3= good; slightly firm
2= fair; moderate softening
1= poor/limp/wilted

Aroma/Odor:
5= typical/strong (already perceptible on intact leaves)
3= typical (perceptible on broken leaves)
1= slight (the odor perception was limited to rubbed and manipulated leaves)

Off-Odor (Persistent; on broken leaves):
5= very strong off-odors/sulfur compounds
4= strong off-odors
3= moderate off-odors (limit of acceptability)
2= slightly off-odors
1= no off-odors

Purchase intent (value for money):
5=definitely would buy
4=probably would buy
3=indifferent
2=unlikely to buy
1=definitely would not buy