



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

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

**Beyond Fresh-Cut: Addressing
Critical Aspects for Fresh
Strawberry Puree**

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Doctorate Course in ‘Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region’ – XXVIII cycle –

Doctoral thesis on “**Beyond Fresh-Cut: Addressing Critical Aspects for Fresh Strawberry Puree**” discussed on **05/05/2016** at the University of Foggia, Italy.

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Dedication

This dissertation is dedicated to my mother Letekal Ghezehey and to the memory of my father Tesfamichael Solomon and sister Saba Tesfamichael.

Acknowledgements

“Trust in the LORD with all your heart and do not lean on your own understanding. In all your ways acknowledge Him, and He will make your paths straight” (Proverbs 3:5-6).

Without the generous financial support by the University of Foggia, (UNIFG) ‘PON-PLASS –Platform for Agro-food Science and Safety’ and OFRALSER projects this would not have been possible. So, my special thanks go to Prof. Maurizio Ricci, rector of the University of Foggia, Prof. Agostino Sevi director of Department of Science of Agriculture, Food and Environment and coordinator of PON-PLASS project and Prof. Giancarlo Colelli (my supervisor, coordinator of the Ph.D. program and the OFRALSER project) for the opportunity. Prof. Giancarlo Colelli inspired and influenced my interest from pure agronomic/horticultural sciences to the postharvest sciences. I am very grateful that his tireless guidance, support, advice during my study period.

Every member of the Postharvest UNIFG group (University of Foggia), especially Prof. Maria Luisa Amodio and Dr. Maria Lucia V. de Chiara are very much acknowledged for continuous help and encouragement, guidance and care. I thank Tecnova Technology Centre (CT Tecnova) –Fundaciòn Tecnova-Company (Almeria, Spain) for hosting and allowing me to work with them during my internship period. Thanks to Dr. Ana Belèn Cabezas-Sarreno and Dr. Mari Carmen Galera for the continuous assistance, guidance and care. All CT Tecnova Foundation personnel are wonderful. My gratitude also goes to all UNIFG community including ‘*A. Di. S.U. Puglia Sede di Foggia*’ for their cooperation, perfect administration, care that was my real how in Foggia. All A.Di.S.U. di Foggia personnel are unforgettable. Without unconditional love and prayers of my parents and friends, I would not have come this far in my life. I am forever grateful to you! Your patience, love, support and encouragement throughout this long endeavor was extraordinary. Finally, I would not find exact words to express my feelings towards my fiancée Semhar Kesete Hagos; your love, trust, and patience are always with me.

“Beyond Fresh-Cut: Addressing Critical Aspects for Fresh Strawberry Puree”

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Abstract

The present study was aimed to address critical aspects related to the development of fresh-blended strawberry purees. The main focus was to evaluate and determine the effects of wounding intensity on respiration rate and biologically active compounds (*Experiment 2.1*), to survey most important strawberry cultivars grown in Italy for fresh-blended purees (*Experiment 2.2*), optimize the blending conditions (time and temperatures) (*Experiment 2.3*), and evaluate and compare packaging conditions using two different plastic materials under active and passive modified atmosphere conditions (*Experiment 2.4*). For this purpose, fresh purees were blended and samples were prepared and physiochemical, nutritional and sensorial attributes were monitored at initial and during storage at 5°C (according to each experiment).

In Experiment 2.1, fruits were submitted to six levels of cut intensity - whole fruit (WHO), 4, 16, 64, and 128 pieces and chopped (CHO) samples. Respiration rate, vitamin C, total phenolic content, antioxidant capacity, total anthocyanin content, total soluble solids, titratable acidity and pH values were evaluated at the processing day (Day 0) and after 2 days at 5 °C (Day 2). Results showed effects of increased respiration rate consequential from cell disintegration of wounded issue up to certain wounding degree (64 pieces cutting), could be minimized significantly at a higher wounding intensity (cutting into 128 pieces and chopped) in strawberry fruits. This resulted in significant increase in total phenolic content, dehydroascorbic acid, antioxidant capacity during storage time with no effects on total anthocyanin content, total phenolic content, pH value and sugar/acid ration content. These results should be considered for processing and packaging optimization of minimally processed strawberries.

For experiment 2.2, fresh puree from six strawberry cultivars ('San Andreas', 'Sabrina', 'Candonga', 'Festival', 'Fortuna' and 'Nabila') were evaluated upon blending. Despite the variability on nutritional attributes, no significant difference on vitamin C content and sensory attributes except sensory color among the studied cultivars was found. Inverse correlation ($r=76$) ($P<0.001$) of total color difference (ΔE^*) and total anthocyanin content, and positive correlation ($r=0.80$; $P<0.001$) of total phenolic content and antioxidant capacity were found. 'Festival' followed by 'San Andreas' and 'Candonga' were mentioned as suitable cultivars for fresh strawberry puree production under optimal blending conditions.

Therefore, to optimize the effects of blending conditions (time and temperature) on quality attributes of fresh-blended strawberry puree, two independent trials - response surface method based on a central composite design (Trial I) and blending temperature (Trial II) were conducted. For Trial I, ten different blending time/temperature experimental runs ranging from 5 to 140 s and from 0 to 21°C respectively were tested. Physicochemical, organoleptic and nutritional quality

attributes were evaluated as responses at the processing day (Day 0) and after 6 days at 5°C (Day 6). All data were fitted to the second-order (quadratic) regression equation. Results showed that at day 0, blending time had significantly negative effect on L* and b* values, titratable acidity and succinic acid, whereas blending temperature had significant influences on total anthocyanin content, sucrose, fructose, and malic acid. After 6 days, however, viscosity, total anthocyanin content, and dehydroascorbic acid were the only parameters found to be significantly affected by the blending conditions. High blending temperature (21°C) had positive effect on total anthocyanin content and negative effect on dehydroascorbic acid content. Therefore, Trial II was aimed to better understand the effect of this factor on certain quality parameters during storage of fresh purees. Strawberries stored at 5 or 21°C overnight were blended at 4000 rpm for 90 and stored for 13 days at 5°C. Color parameters vitamin C, total anthocyanin content and sensorial attributes were monitored after 0, 3, 8 and 13 days at 5°C. A two-way analysis of variance and Tukey's test ($P < 0.05$) showed that after 3 days, samples blended at the lowest temperature showed significantly better color maintenance (lower ΔE^*), aroma and overall acceptance and stable ascorbic acid content. In general, blending time less than 90 s and temperature 5 °C can be optimal blending conditions with improved packaging conditions.

Finally, Experiment 2.4, was aimed to evaluate and compare two packaging materials, namely polypropylene/ethylene vinyl alcohol (PP/EVOH) and polypropylene/polyamine (PP/PA), both under active (5% O₂+13% CO₂) and passive (air) modified atmosphere packaging (MAP), to package fresh-blended strawberry purees. Physicochemical, sensorial, nutritional attributes and spoilage microbial counts were evaluated after 0, 2, 6, and 12 days of storage at 5°C. A two-way analysis of variance for treatment, storage time and their interactions was run and significant means were separated by Tukey's test ($P < 0.05$). Results showed that after 12 days at 5°C, packaging significantly affected the physical and nutritional attributes and microbiological counts. Samples in active MAP in PP/EVOH or PP/PA maintained slightly higher nutritional and sensorial attributes during storage. After 12 days at 5°C, samples in active PP/EVOH had lower microbiological counts ($< \text{Log } 4 \text{ CFU g}^{-1}$) and no perceived off-flavor compared to the other treatments. In conclusion, fresh-blended strawberry purees can be stored for about 12 days at 5°C using active PP/EVOH MAP condition for quality maintenance and freshness.

As a final remark, high convenience fresh-blended strawberry puree can successfully be developed without significant losses of nutritional, organoleptic attributes for ready-to-use with 100% fruit product puree. The influences of pre-harvest factors on quality and flavor profiles and non-thermal processing techniques should be considered for future studies.

Key Words: Anthocyanin, antioxidant capacity, ascorbic acid, central composite design, cutting degree, dehydroascorbic acid, 'Festival', Fresh puree, respiration rate

PART ONE: GENERAL

1. General Introduction

1.1 Convenience product based on fresh fruit and vegetables: types and market data

Convenience, together with price, sensory appeal and health-related concerns, is believed to be an important determinant of food choice (McIntosh, 1996). Like healthiness or sensory quality, convenience is a broad, multidimensional construct, in which not only the foods' characteristics but also those of consumers and circumstances play a role (Costa *et al.*, 2007). Although it is not easy to find a clear and simple definition of "convenient food products", "any food which has had work performed on it outside the home can be regarded as convenience foods" (Scholliers, 2015). Referring to the basic fact that convenience foods make life easier, not just for those preparing a meal, but also for those eating it (untied by place, time or company). Brunner *et al.* (2010), defined convenience food products as "those that help consumers minimize time as well as physical and mental effort required for food preparation, consumption and clean-up". Convenience determines where, when, why, what, how and even with whom we eat (Costa *et al.*, 2007). Currently, the concept of convenience foods expands according to the 'modernization' of the food chain and a much more rapid pace than before (Scholliers, 2015), including also the convenience for safe and high quality and handling suitability.

Nowadays, consumers' ongoing demand for convenience and healthy foods has kept consumption of fresh-cut fruits and vegetables growing (Moreira *et al.*,

2015). Consumers increasing demand for time-saving and healthy products such as fresh-cut fruits and vegetables that can be used without spending much time for preparation (Boča *et al.*, 2014). The convenience food may also include the products beyond the fresh-cut products such as “minimally processed” food products (those that in Italian are known as *V gamma*). Most of convenience food products need little or no further preparation before being consumed and can be used at any time, quickly and easily as by thawing or heating. This may enable the consumers to save time and effort in food activities, related to shopping, meal preparation and cooking, consumption and post-meal activities (Buckley *et al.*, 2007; Daniels *et al.*, 2015). Fresh-cut products in a raw state and even though minimally processed, remain in a fresh state, ready to eat or cook (Lamikanra, 2002). Personal time is a more valuable and fresh-cut products satisfy a basic need to have a fresh product ready for consumption and do not require further washing with comparable quality in terms of nutritional values to the traditional fruits and vegetables prepared at home. Therefore, the fresh-cut scenario in 2015 highlights a consolidation and further acceleration of the market growth in 2016. The benefits and enforcement of the newly introduced Law 77/2011, resulted in the full transfer consumers of more guarantees in terms of safety and quality of the product (Nielsen, 2016).

Some of the popular convenience/processed food products based on fruits and vegetables are packaged chips, canned vegetables, bread, commercialized fruit juices, salt, sugar, flour, frozen meals/pre-packaged foods, based on type, the

market of convenience fruits and vegetables includes single fruit or vegetable and other preserved fruits and vegetables using sugar, vinegar, acetic acid, jams jellies and mixed pickles. Moreover, these can also be fresh, fresh-cut, canned, frozen, dried and convenience (Marketsandmarkets, 2016).

However, the so called ‘convenience food products’ which are not originated from fruit or vegetables sources may not be so suitable for the human health because most of them are over-processed and may not provide enough nutritional values and sometimes have excessive amounts of sodium, sugar and saturated fats. Many of the health conditions like heart disease, hypertension, or diabetes are related to the foods. Pre-cut, prewashed, frozen, and canned fruits and vegetables can also be classified as convenience foods. Fruits and vegetables are essential components of the human diet and there are considerable evidences of the health and nutritional benefits associated with their consumption (Ramos *et al.*, 2013). The changes in life-style and awareness for healthy convenience, is related, in a balanced diet, to the presence of a wide variety of minimally processed fruits and vegetables has been developed. A diet high in fruits and vegetables has a significant protective effect against the risk of various cardio metabolic diseases, including hypertension, stroke, diabetes and peripheral arterial diseases because fresh fruits and vegetables are major source of essential vitamins and minerals, such as vitamin A, vitamin C and potassium, needed for human wellbeing. These perishable living products require coordinated activity

by growers, storage operators, processors and retailers to maintain quality and reduce food loss and waste (Mahajan *et al.*, 2014).

Therefore, the trend for convenience products is based on fresh fruits and vegetables. While enhancing convenience, the quality and shelf life of fresh fruits and vegetables may be compromised. In some cases, may require addition of preservatives or use of highly impacting processing technologies. Most of them may not be free of preservatives, unnatural coloring, flavoring, and other appetizing substances. Numerous conventional thermal processing techniques such as blanching, pasteurization and sterilization, thermal drying, preservatives and novel thermal and non- thermal processing techniques (i.e. dense phase carbon dioxide, pulsed electric field, ozone processing, ultrasound processing, high hydrostatic pressure processing, radiation processing) can be implemented as processing measures (Rawson *et al.*, 2011). Since, quality of fresh fruits and vegetables should not be compromised for just conveniences; high quality convenience products fulfill the modern consumers' demand. Figure 1 illustrates the relationship between convenience, quality and shelf life of fresh foods. Concern about naturalness, nutrition knowledge, and cooking skills were identified among the strongest predictors of convenience food consumption among others (Brunner *et al.*, 2010). Consumer profile is changing as an effect of a stressed lifestyle. The number of workers and singles is gradually increasing; they are short on time and demand minimally processed foods (ready-to-use) to save time on food preparation. Ready-to-use or to-eat products should have an

adequate shelf-life; it should be at least 4–7 days or even 21 days, depending on the products (Corbo *et al.*, 2015).

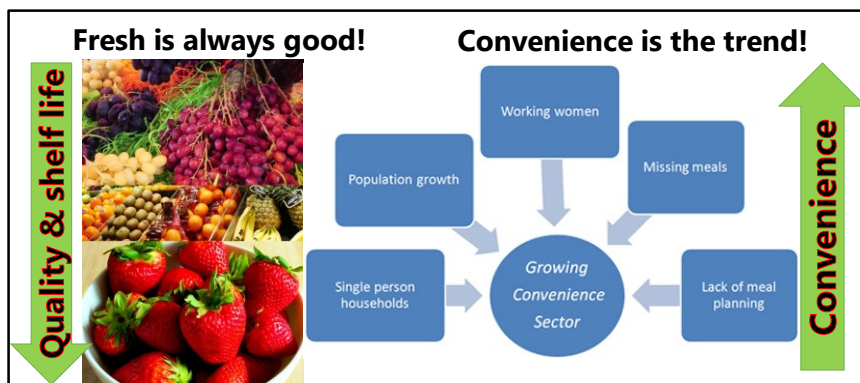


Figure 1 Quality vs conveniences: (Adapted from Freedman (2011))

Therefore, consumers aware of the modern trends in food industry express concerns regarding extended shelf -life and prefer the food products produced hygienically, safely and with a known origin/source of raw material. Convenience foods must be tasty and high quality, while meeting consumer expectations in terms of ease of use, safety, variety, packaging, nutritional value and product appeal and all season availability. Convenience products based on fresh fruits and vegetables probably meet these requirements as the intake of fruits and vegetables has been associated with a notable health-protecting factor against diseases caused by oxidative stress, including coronary heart disease, cancer, and neurodegenerative pathologies (Fu *et al.*, 2011; Morales-Soto *et al.*, 2014).

Fresh cut products possess the same nutritional value, consistent quality and freshness as an intact fruit hence facilitating the consumer by adding to the convenience and reducing storage space as all packages include only edibles (Artés *et al.*, 2007). This may enhance the number of consumers recognizing fresh-cut products as healthy convenience products.

A recent market size report on fresh-cut (convenience) fruits and vegetables showed that both volume of production and values by region is slowly growing (Marketsandmarkets, 2016). The global market size by region (2012-2019) in terms of volume of production and values for 2012-2013 estimations for 2014 and projects up to 2019 was reported. According to this study the global volume of production of fresh-cut fruits and vegetables grew from 5,259.5 MT to 5,879.3 MT from 2012-2014 and is projected to reach 7,777 MT in 2019 which was valued for 6,418.7 million Euros in 2012, 7,178.1 million Euros in 2014 and is expected to be valued to 9,505.0 million Euros in 2019. The major contributing regions of the world are North America, Europe and Asia-Pacific with a growing contribution from Latin America and less from rest of the world including Africa. Current research of postharvest sciences has successfully discovered the remedies to the technical issues that caused hurdles in the industrialization of fruits and vegetables hence expanding ground for the fresh cut sector (Oms-Oliu and Soliva-Fortuny, 2011). Increased innovation through new product development, adoption of appropriate distribution chains, novel preservation treatments and advanced packaging strategies are the heart of contemporary research in order to

enhance the growth at the same rate to boost fresh cut industry (Martín-Belloso and Soliva-Fortuny, 2011), There is a broad scope of research for the development of technologies that can provide practical solutions for shelf life enhancement of fresh-cut fruits and vegetables equally ensuring their sensory qualities, microbiological safety and nutritional value.

Fresh-cut products satisfy the consumer demand for easy-to-use, convenient, healthy food (Francis *et al.*, 2012). Apart from this, most of the convenience food products in market are highly processed and may not be free of additives. Even the products based on fruits and vegetables on the market are greatly processed as clear juices, nectars, jams and preserves undergoing through high processing and preservation temperatures, during pasteurization, sterilization, blanching. As a result, undesirable changes in their organoleptic features (flavor, color, texture) and often in considerable losses in their valuable bioactive components, such as vitamin C and polyphenols, including anthocyanin pigments may happen (Marszalek *et al.*, 2015).

This is particularly important with soft berries such as strawberries. Not only due their attractive color and flavor, but also due to the fact that among fruits, fresh strawberries and products based on fresh fruits have very short shelf life span (Sulaiman and Silva, 2013), high phytochemical content (Alvarez-Suarez *et al.*, 2014). Hence no wonder that strawberry is one of the most important fruit both economically and nutritionally as fresh and minimally processed products available in the market.

1.1.1 Strawberry fruit basics: importance, main destinations, new products

Strawberry (*Fragaria x ananassa* Duch.) is one of the most economically important cultivated berry fruit crops worldwide with more than 7.5 million MT produced in 2013 (FAOSTAT, 2015). Strawberry is among the most widely consumed fruits in the world and its worldwide production ranks second from berries after the grape. In addition, being an attractive fruit due to its color and flavor, strawberries are consumed both as fresh fruit, as processed and as an important source of antioxidant compounds including anthocyanins, flavonoids, phenolic compounds and nutrients (Henriqueta *et al.*, 2014). Strawberry is considered as a good source of antioxidants, mainly given to its high vitamin C and phenolic contents. Phenolic classes commonly found in strawberries are hydroxybenzoic acids (gallic and ellagic acids), hydroxycinnamic acids (p-cumaric), hydrolysable tannins (ellagitannins), flavonols (quercetin, kaempferol and myricetin), flavan-3-ols (catequins, epicatechins), and anthocyanins (quantitatively the most important type of polyphenols in strawberry) being pelargonidin-3-glycoside, the most important flavonoid pigment (Tulipani *et al.*, 2008; Pineli *et al.*, 2011). The high levels of micronutrients and phytochemical compounds in strawberries, exhibit functional roles in plant growth and metabolism and are essential for the nutritional and organoleptic qualities of the fruit (Tulipani *et al.*, 2008). Currently, due to the multiple preventive and therapeutic health benefits associated with the consumption of strawberries are considered as a “functional food” (Fernández-Lara *et al.*, 2015). Consequently,

strawberry is consumed both as fresh or processed forms. Large quantities of strawberry production (approximately 80%) is for fresh market, while the rest are intended for industrial processing purposes, such as the production of yogurts, jams, jellies, dessert toppings (Šamec *et al.*, 2016).

Table 1 The top five Strawberry producing countries and total production and percentage on the world total production (2015); FOASTAT data, September 2015.

Rank	Country	Strawberry production 2013 (MT)	% of world total
1	China	2,997,504.0	38.7
2	USA	1,360,869.0	17.5
3	Mexico	379,464.0	4.9
4	Turkey	372,498.0	4.8
5	Spain	312,500.0	4.0

Sources: (FAOSTAT, 2015) (last accessed by Top 5 of Anything: September 2015).

According to the FAOSTAT (2015) report, in 2013, China with 39% market share and about 3 million MT was the first strawberry producing Country in the world, while the USA took the second place with 18% of the share. Third place went to Mexico with 5%, followed by Turkey and Spain as fourth and fifth places (Table 1). Italy is out of the top 10 representing just over 0.5% of world production. According to Italiafruit News (2016), during the last a few years (2000-2014), Spain and Poland were the largest producers of strawberry in Europe, with a significant increase (+85%) of strawberry production reported in Spain. In Italy, the export market of strawberry showed a reduction (-43%)

(Italiafruit News, 2016), mainly because of increased domestic consumption. In 2014, the Italian strawberry production showed a slightly decrease in production (as equal as 2011). However, about 4% increase in strawberry consumption was found in 2014 compared to the previous years and still expected to grow. The expansion of production and marketing season and the availability of new varieties were among the driving forces for this increase. Strawberry production showed variability in the different regions of Italy. In South Italy, a significant reduction in Calabria (-20%) and Sicily (-5%) but not in Basilicata that had an increase in production (+9%). In the north, also a fall in Emilia Romagna (-6%), in the provinces of Bolzano (-12%) and Trento (-5%) but not in the Piedmont (+13%) (Italiafruit News, 2016). Strawberries are an important fruit in the Mediterranean diet because of their high content of essential nutrients and beneficial phytochemicals, which seem to exert beneficial effects in human health (Alvarez-Suarez et al., 2014).

1.1.2 Postharvest physiology and technology of fresh and fresh-cut strawberries

Strawberries are non-climacteric fruit showing no increase in respiration rate or ethylene production during ripening (Merchante *et al.*, 2013). Strawberries should be harvested near to full ripe usually when 75% of the berry surface of fruits showing red or pink color (Mitcham *et al.*, 2000). Strawberry fruits harvested at their best stage of development (highest weight) and ripening (highest sugar: acid ratio, with appropriate color and texture) for consumption. Usually it takes ~30 days from fruit set to achieve full size and ripening. However, this time is highly dependent on light, temperature, soil composition, and other conditions of cultivation (Cordenunsi *et al.*, 2002). In order to have the highest quality in terms of flavor, taste and color strawberry must be harvested at full maturity. Softening of the fruits as they ripen involves thinning of cell walls and liquefaction of cell contents (Szczesniak and Smith, 1969). The large cells and thin cell walls in strawberry fruits contribute to their high level of susceptibility to mechanical damage (Aliasgarian *et al.*, 2015). During ripening, changes may result in texture and decrease their quality and postharvest life very rapidly. According to Mitcham *et al.* (2000), quality indices of fresh strawberry fruits based on recommended appearance (color, size, shape, freedom from defects), firmness, flavor (soluble solids, titratable acidity and flavor volatiles), nutritional value (vitamin C) should be considered. As an example, for acceptable flavor, a minimum of 7% soluble solids and/or a maximum of 0.8% titratable acidity are recommended. The ripe fruits usually have a short postharvest life

estimated 1-2 days at room temperature and around 5 days under cold-stored (0-4 °C) (Martinez *et al.*, 2008; Aday and Caner, 2014; Henriqueta *et al.*, 2014; Jouki and Khazaei, 2014). Temperature management remains the most important factor to reduce the losses. Fresh strawberries can optimally be stored at low temperature and high relative humidity (RH) (0 ± 0.5 °C; 90-95 % RH). Strawberries are among the highly respiring fruits ranging from 6 to 10 ml CO₂ kg⁻¹h⁻¹ (at 0 °C) to 50 CO₂ kg⁻¹h⁻¹ (20 °C) (Mitcham *et al.*, 2000). Since there are no postharvest fungicides allowed on strawberries rapid marketing or quick cooling, storage at 0 °C, preventing fruit injury, and shipment under high carbon dioxide are the best methods for disease control (Mitcham *et al.*, 2000). Furthermore, care separation of diseased or wounded berries from the healthy berries at harvest may minimize contaminations. Botrytis rot (Grey mold) caused by *Botrytis cinerea* that continues to grow even at 0 °C and rhizopus rot caused by the fungus *Rhizopus stolonifera* that does not grow at temperature below 5 °C are major concerns for postharvest losses of strawberry (Romanazzi *et al.*, 2001). Temperature management is the simplest method of control.

When strawberry fruits are stored at low temperature, their shelf-life can be extended to at least one week. Nevertheless, the delay between harvest and storage at the proper temperature is critical for the success of the treatment. It was observed that fruits stored at low temperature, 6 h after harvest, showed undesirable changes in color and texture and also a reduction of around 50% of water content in comparison to those that were immediately cooled after harvest

(Nunes *et al.*, 1995). The damaging changes in sensorial and nutritional values of strawberries preserved in cold storage is significantly reduced.

Several research works have aimed to find the best compromise between extended shelf-life and maintenance of nutritional value. Modified atmosphere, which can be produced by increasing CO₂ level while reducing O₂, has yielded good results regarding strawberry preservation (Cordenunsi *et al.*, 2003). Cold storage is an efficient way to preserve strawberries, since no deleterious changes were observed either sensorial or nutritional values. The beneficial effect of controlled and modified atmospheres in extending the storage life and delay decay of strawberries have been well documented. (Gil *et al.*, 1997) observed that storage in elevated CO₂ atmospheres, a commercial treatment for postharvest decay control, induced a paleing or 'bleaching' of the internal flesh color of 'Selva' strawberry. (Perez and Sanz, 2001) worked for the enhancement of strawberry firmness along with deterioration of fungal growth using high CO₂ and high O₂ atmospheres. Titratable acidity, color, sugars, distribution of organic acids and off odors were observed after one week of storage in four different atmospheres, two with high O₂ concentrations i.e. air and 90% O₂ and two with high CO₂ and high O₂ concentrations (20% CO₂ + 5% O₂ and 80% O₂ + 20% CO₂). It was concluded that the atmospheres with the high CO₂ concentration found high contents of off flavor. In another study by Holcroft and Kader (1999) observed that during a 10-day storage time of strawberries the anthocyanin concentrations increased but this rise was slower in the air mixed with 10-20%

CO₂. In the CO₂ enriched in air, the flesh redness was also less as compared to storage in air. Off-flavor development is associated with increased production of ethanol and acetaldehyde. The alcohol content of strawberry fruits increased with the length of storage and with higher concentrations of CO₂ (Thompson, 2010b). ‘Pajaro’ strawberries stored in air, or air with 10, 20 or 30% CO₂ for 5 days at 5°C, followed by an additional 4 days in air at the same temperature. Ethanol and acetaldehyde accumulation was very slight, although sensory evaluation of the fruits showed that off-flavor were present at transfer from CA, but not after the following storage in air (Colelli and Martelli, 1995).

Another important type of stress in strawberry is mechanical damage that occurs during harvesting and postharvest manipulation of fruits. This stress is accompanied by physiological and morphological changes that affect the fruit commodity. Apart from the mechanical stress, there are other types of stress due to biological and environmental factors, which also cause quality reduction (Aliasgarian *et al.*, 2015). Strawberries are extremely perishable because of the high respiration rate and weight loss, bruising and physical injury due to the soft texture and lack of protective peel during handling, transportation and storage (Henriqueta *et al.*, 2014).

Minimally processed strawberry or strawberry-based new products attracted the interest of food industries during the last few decades. The quality and shelf-life of fresh-cut or even fresh strawberry-based products can be influenced by several pre and post harvesting factors among which are the cultivar, processing and

storage and packaging conditions. During processing, several complex interrelationships among the effects of wounding on physiological processes occurs in fresh-cut products (Saltveit, 1997). Wound induces signals that provoke physiological and biochemical responses both in the adjacent and distant tissues; several of these responses are detrimental to the quality and shelf-life of fresh-cut produce. The immediate response to wounding include increase in respiration rate and ethylene production which may be interrelated to the stimulation phenolic metabolism and wound healing responses. Furthermore, these processes can potentially be a source of poor flavor, softening, browning and toughening of fresh-cut products (Figure 2) (Saltveit, 1997).

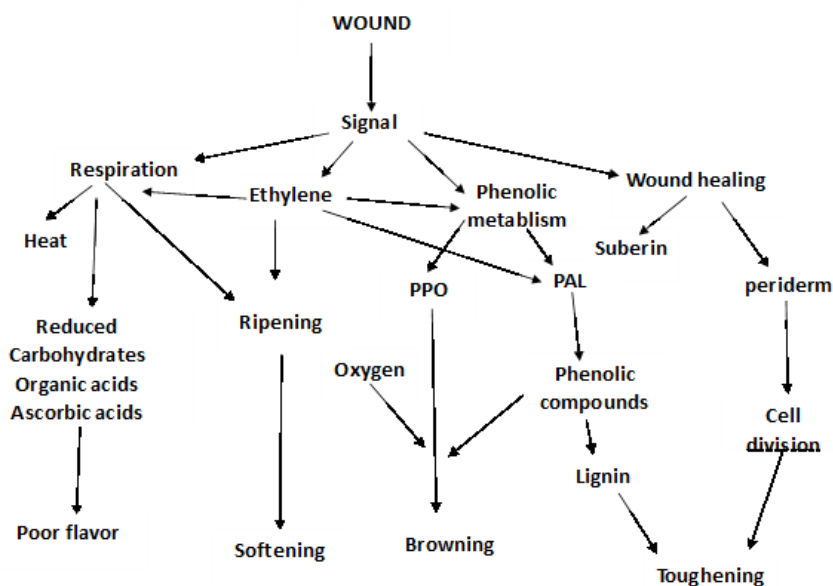


Figure 2 Interrelationship among the many effects of wounding on physiological processes in fresh-cut fruits and vegetables adapted from Saltveit (1997).

Wounding and its responses to degree of damages vary depending on species, cultivar, maturity, storage or processing temperature, cutting protocols, including sharpness of the cutting, and temperature at which the cutting is done (Lester, 2003; El-Ramady *et al.*, 2015). The response to wounding may vary depending on several factors such as genotype or cultivar, crop conditions (environmental and cultivation techniques), ripening season, harvest and postharvest conditions, including processing (Olsson *et al.*, 2004; Giampieri *et al.*, 2012). Cultivar screening is an important step in the study of new product based on fresh fruits, because it allows to identify genotypes which better respond to postharvest handling and minimal processing (Cabezas-Serrano *et al.*, 2009). Appropriate processing, storage and packaging conditions are therefore crucial steps in facing the challenges during production and marketing of fresh minimally processed product in order to slow down the rapid quality deterioration and reduced shelf-life (Hussein *et al.*, 2015).

Despite an increased interest for the food industry in developing new fresh-blended/squeezed fruit juices and purees that are not very different from those fresh smoothies made at home, as far as we know no work has been done on fresh-blended strawberry purees. Fresh-blended purees are expected to maintain the nutritional and organoleptic characteristics of fresh fruits but also suitable from technological point of view.

This may add more value when minimally products are prepared to fulfil current demands of consumers regarding to the health and diet on top of the ease of use, handle or consume. Therefore, developing a new convenience product based on fresh strawberry fruits, may have a significant influence on the consumption. This can prove new opportunities towards improvement of cultivars for superior quality and technologically suitable not only for fresh and fresh-cut products but also new and more convenience products based on fresh strawberries. This becomes more important when the raw material for such convenience products come from low impact and organic farming systems and yet blended with no chemical additions.

1.2 General and specific objectives

Strawberry as a fruit and its value added products are highly demanded in the market with consumer acceptance based on many factors of cardinal significance including color, flavor, nutritional content, organoleptic properties, antioxidant capacity etc. Fresh strawberry purees are one of the products that can be introduced as a ready to use item into the market with expectations of positive acceptance if the nutritional, organoleptic and technological aspects are suitably addressed. Developing new convenience product based on fresh strawberry fruits with guaranteed quality and safety for at least 10-12 days of refrigerated storage without any thermal or chemical treatment and additives, is an interesting gap between the fresh-cut and processed food products. For the maintenance of optimum nutritional, organoleptic and technological aspects of fresh purees, they need to be minimally processed. Therefore, it is important to identify the factors that contribute towards the degradation of the nutritional and organoleptic attributes of added value food products prepared from strawberries. The general objective of the study was addressing these critical aspects for developing fresh strawberry purees focusing on main factors which may influence its quality attributes. To this aim, specific objectives were therefore the following:

- i. to determine the effect of wounding, and of its intensity, on physiological and quality changes of fresh strawberry fruits, with particular focus on the respiration rate and on the fate of biologically active compounds;

- ii. to survey strawberry cultivars grown in Italy for quality and technological attributes of fresh-blended puree in order to evaluate the extent of variability for each attribute, define differences and common characteristics, and, possibly, identify those which are better suitable to be used as a raw material for fresh- blended puree production;
- iii. to optimize the blending conditions (i.e. blending time and temperature) in terms of quality and shelf-life of fresh strawberry purees;
- iv. to study and compare the effect of different packaging materials on quality and shelf life of strawberry purees.

PART TWO: EXPERIMENTAL

2.1. Effect of wounding intensity on physiological and quality changes of strawberry fruits

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Abstract

Fresh-cut fruits are more perishable than whole fruits due to the physiological stresses caused by physical damage or wounding. Wounding (cutting) is the most important step in fresh-cut processing. The effect of wounding intensity on physiological and quality changes of fresh-cut strawberries was investigated. The focus was to determine the impact of wounding on respiration rate and nutritional quality changes of fresh-cut 'Candonga' strawberries. Fruits were submitted to six levels of cut intensity - whole fruit (WHO), 4, 16, 64, and 128 pieces and chopped (CHO) samples. In this study, respiration rate, vitamin C, total phenolic content, antioxidant capacity, total anthocyanin content, total soluble solids, titratable acidity and pH values were evaluated at the processing day (Day 0) and after 2 days at 5 °C (Day 2). Results showed that wounding intensity significantly influenced the respiration rate, ascorbic and dehydroascorbic acids, total phenolic content, and antioxidant capacity. Respiration rate increased with wounding intensity up to the level of 64 pieces ($18.2 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) compared to WHO ($10.0 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and then decreased in the CHO samples ($5.1 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$). At Day 2, the stress caused by the high intensity of cutting (64 pieces and CHO) induced a higher degradation of ascorbic acid, total phenolic content, and

antioxidant capacity and the increase of dehydroascorbic acid. No significant difference was found in total soluble solids, pH-value and titratable acidity and total anthocyanin content. In general, wounding significantly influenced respiration rates and nutritional quality. The changes related to the stress seem to first increase with the degree of cutting up to a certain intensity (64 pieces) and then decrease when the damage to the cells was very high to compromise their functionality. These results should be considered for processing and packaging optimization of minimally processed strawberries.

Key words: Cutting degree, 'Candonga', fresh-cut, respiration rate, ascorbic acid, dehydroascorbic acid, total phenolic content

Introduction

Fresh fruits are living tissues subject to continuous physiological activity inducing compositional and structural changes leading to ripening and/or senescence. These reactions cannot be stopped, but can be delayed within certain limits to prolong the shelf life of fruits as much as possible (El-Ramady *et al.*, 2015). Fresh-cut fruits are usually more perishable than whole fruits because they have been subjected to physiological stresses caused by physical damage during processing, leading to several physical and physiological changes such as increase in respiration rate. Wound-induced immediate response may include a wound signal formed in adjacent and distant tissues, which elicits a wide range of physiological and biochemical responses. The most common may include an increase in respiration rate, ethylene production, quality changes, synthesis

and/or loss of phytochemicals, decreasing of nutritional value, enzymes activity and microbial spoilage (Brecht, 1995; Surjadinata and Cisneros-Zevallos, 2003). The complex interrelationship among the several effects of wounding on physiological processes of fresh-cut produce can be found in Saltveit (1997). Wound responses vary depending on species, cultivar, maturity, storage or processing temperature, cutting protocols, including sharpness of the cutting tool, and temperature at which the cutting is done (Lester, 2003; El-Ramady *et al.*, 2015), O₂ and CO₂ levels, and water vapor pressure (Brecht, 1995). The wound-induced changes within the wounded tissues involve altered expression of several genes and changes in enzyme activities (Mehta *et al.*, 1991) which may be directed to healing the damaged tissue and providing defense mechanisms that prevent further damage (Chung *et al.*, 2006).

During storage, a higher rate of respiration usually leads ageing of the products by using reserve energy during oxidative-reduction process. The higher the rate of respiration, the shorter will be the shelf life. Peeling and slicing increase respiration rate up to 3 folds in baby carrot (Simões *et al.*, 2011), and 2-folds in kiwi fruit. However, the rate of respiration remained unaffected after peeling and slicing in ripe bananas. This seems to differ between climacteric and non-climacteric fruit and with the physiological age of climacteric fruit (Gunes and Lee, 1997). Moreover, fresh-cut processes induce an overall increase in the activities of phenylalanine ammonia-lyase (PAL) and often peroxidase (POD) and polyphenol oxidase (PPO) in response to cutting (Saltveit, 2005). Post-

cutting interaction of product substrates with enzymes, such as ascorbate oxidase, PPO, and POD, may enhance degradation of phytonutrients. In addition, browning due to oxidation of phenols may reduce nutrient content and often results in degradation of color, texture and flavor of the product (Saltveit, 1997; Francis *et al.*, 2012). Various compounds (e.g., antioxidants, calcium) and treatments (e.g., low oxygen, heat-shock) reduce wound-induced tissue browning by interfering with either the synthesis or oxidation of precursor of phenolic compounds (Brecht, 1995; Saltveit, 1997). According to Francis *et al.* (2012), an increase in nutritional value of wounded tissues because of the induced synthesis of phenolic compounds has been reported for cut lettuce, celery, carrot, parsnips, and sweet potato, while in the same study a decrease of phenols was observed in cut zucchini, radish, potato, and red cabbage. Each product type has a unique combination of compositional factors affecting the amount and profile of wound-induced soluble phenolics. Their concentration is also dependent on the type of tissue, initial levels of reduced ascorbic acid and soluble phenolic compounds. Even reduced ascorbic acid resulted to be greatly affected by wounding (Reyes *et al.*, 2007).

Mechanical damage is considered as a type of stress that occurs during the harvest and postharvest handling of fruits. The soft and thin strawberry tissues (large cells and thin cell walls) contribute to their high level of susceptibility to mechanical damage such as abrasions, cuts, bruising and juice leakage (Kader, 1991). Maximum damage index (more than 50%) was related to the picking stage

although packing and delivery to the market were also important in strawberry (Aliasgarian *et al.*, 2015). Moreover, the time between harvest and initial mechanical damage is an effective factor in susceptibility of fruits to the damage (Martinez-Romero *et al.*, 2004). They reported that increasing the time led to decreasing the turgidity of young tissues and finally raised their resistance to damage. Results indicated that the variety, operation stage, fruit position in the box, and box position on the truck, had significant effects on the extent of the fruits mechanical damage (Martinez-Romero *et al.*, 2004).

In the recent years the food industry is interested in strawberry-based new products as source of bioactive compounds with higher antioxidant capacity, as strawberries are known to be a good source of vitamin C, anthocyanins and flavonols and, among fruits, one of the highest in term of antioxidant activity (Cordenunsi *et al.*, 2005). Understanding the stress-induced changes is important in order to develop reliable approaches to control the stress responses (Hodges and Toivonen, 2008), and improve the quality of minimally processed products. This is particularly motivating to investigate the effect of wounding on soft and fresh fruits like strawberry. Therefore, the main objective of the present study was to determine the effect of wounding, and of its intensity, on physiological and quality changes of fresh strawberry fruits, with a particular focus on the respiration rate and nutritional compounds.

Materials and methods

Sample preparation and analyses

‘Candonga’ strawberries (*Fragaria x ananassa* Duch.) were purchased from local stores in Foggia (South Italy) and stored at 5 °C overnight. In the next morning, fruit with uniform color and size, free of physical defects and decay were grouped into six sample categories (treatments). For each group, samples (approximately 600 grams) were subjected to six different wounding intensities: Whole fruit (no cutting), cutting into 4, 16, 64, 128, pieces and chopped defined as WHO, P4, P16, P64, P128 and CHO, respectively (Figure 1.1). About 12-15 fruit were cut (300 g) for each replicate and used for initial determinations; whereas the remaining samples were stored at 5 °C under a continuous flow of humidified air for 2 days. Respiration rate, total vitamin C, total phenolic content, total anthocyanin content and antioxidant capacity, total soluble solid content, pH value, titratable acidity and sugar/acid ratio were evaluated initially at the processing day (Day 0) and after storage (Day 2). All samples were prepared and analyzed in triplicates.

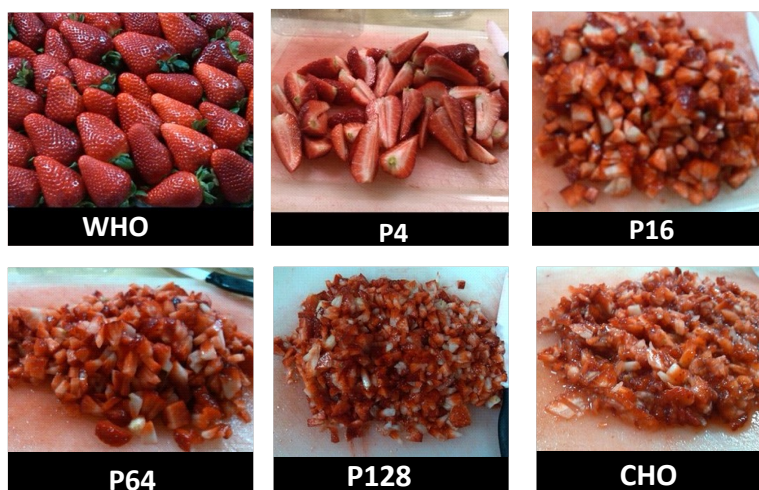


Figure 1.1 Fresh-cut 'Candonga' strawberries subjected into different wounding intensities (WHO, P4, P16, P64, P128 and CHO representing for whole fruit, cut into 4, 16, 64, 128 pieces and chopped).

Respiration rate

Respiration rate of fresh-cut strawberries was measured in static conditions as described in Kader (2002a). Respiration rate was determined at 120 min after cutting. A triplicate sample (about 300 g) of strawberry tissues were placed in 5 L sealed glass jars with a plastic septum for sampling gas. The jars were initially left open in the cold room (5 °C); after closing, gas samples (0.5 mL) were collected from each jar after the required time to accumulate CO₂ in the headspace up to a concentration of 0.1-0.2%, and injected into a gas chromatograph (Shimadzu, model 17 A, Kyoto, Japan) equipped with a thermal conductivity detector (200 °C). Separation of CO₂ was achieved on a Carboxen 1006 plot (30 m × 0.53 mm, Supelco, Bellefonte, PA, USA), with a column flow of 7 mL min⁻¹, and an oven temperature of 180 °C. Calculation of respiration rate

was based on the difference in concentration, the sample weight, the free volume inside the jar, and the elapsed time, as in equation (1):

$$y_{CO_2} = y_{CO_2}^i - \frac{R_{CO_2} W}{V_f} (t - t_i) \times 100 \quad (1)$$

Where $y_{CO_2}^i$ and y_{CO_2} are CO₂ concentrations (percentage) at the initial time t_i (or time zero) and at time t (h) respectively, R_{CO_2} is respiration rate in mL kg⁻¹ h⁻¹, W is the total weight of the product (kg), and V_f is the free volume inside the jar (mL), which is the total volume of the glass jar minus the volume occupied by the sample, assumed equal to the apparent density of sample (e.g. strawberry fruit approximately 1.0 g cm⁻³) (Caleb *et al.*, 2012a).

Compositional attributes

Vitamin C content was measured in 5 grams of homogenized strawberry tissue as L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA) contents (expressed as mg of AA, DHA) or total vitamin C (AA + DHA) per 100 g of fresh weight (mg 100 g⁻¹ FW) following the procedure by Zapata and Dufour (1992) with slight modifications.

Total phenolic content (TPC) was analyzed using the Folin–Ciocalteu method of Singleton and Rossi (1965), with some modifications where five grams of tissue were homogenized in an Ultraturrax (IKA T18 basic, Wilmington, NC, USA) with 10 mL of extraction buffer containing 200 mL of distilled water, 800 mL of methanol and 2 mM (84 mg L⁻¹) of sodium fluoride (NaF). Extracts were then centrifuged at 4 °C and 10000 rpm for 5 min. Extract of 100 µL was mixed with 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL

of sodium carbonate solution (200 g L^{-1}). Then samples were left at room temperature for 2 h in the dark. The absorbance was read at 725 nm against a blank (prepared in the same way except the 100 μL of sample was replaced by 100 μL distilled water) using a spectrophotometer (UV- 1700 Shimadzu, Jiangsu, China). TPC was calculated based on the calibration curve of gallic acid and results were expressed as milligram gallic acid equivalents per 100 g fresh weight ($\text{mg GAE } 100 \text{ g}^{-1} \text{ FW}$).

Total anthocyanin content (TAC) was determined following the protocol described by Cordenunsi *et al.* (2002), with small modifications. One gram of strawberry tissue was mixed with 20 mL of extraction solution (methanol in 1% hydrochloric acid; HCl-MeOH mixture) and homogenized for 20 s using Ultraturrax (IKA T18 basic, Wilmington, NC, USA). The homogenate was then centrifuged for 15 min at 4°C at 2000 rpm. At that time, 700 μL of supernatant plus 300 μL of 1% HCl-MeOH solution were put in cuvettes and absorbance was read immediately in a spectrophotometer at 510 nm. Results were expressed mg of pelargonidin-3-glucoside equivalents per 100 g of fresh weight ($\text{mg PG-3- glu } 100 \text{ g}^{-1} \text{ FW}$).

The antioxidant capacity (AC) assay was conducted on the same extract made for TPC, following the method of Brand-Williams *et al.* (1995), with slight modifications. Fifty μL of diluted extract was pipetted into 950 μL of DPPH (2, 2-Diphenylpicrylhydrazyl) solution and absorbance were read after 24 h at 515 nm. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was

used as a standard and results were expressed in milligrams of Trolox equivalents per 100 g of fresh weight (mg TE 100 g⁻¹ FW).

Total soluble solids (TSS) were obtained by measuring the refractive index of strawberry juice using a digital refractometer (Atago RX-7000cx; Atago Co. Ltd., Japan) at 25 °C. One gram of sample was used to determine the pH and titratable acidity (TA), with an automatic titrator (T50 M Terminal, METTLER TOLEDO, Switzerland) against a volume of 0.1 N NaOH until reaches the final pH of 8.2. TA was expressed as g of citric acid equivalent per 100 g fresh weight (g citric acid 100 g⁻¹ FW). TSS/TA ratio was calculated from by dividing TSS by TA.

Statistical analysis

Data were subjected to a two-way ANOVA (for treatment and sampling time), treatment means were separated by Tukey's test at $P < 0.05$ (5% significance level) using Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA USA) software.

Results and discussion

Wounding intensity had significant effect on most parameters except pH; the same for storage time with exception of total phenolic content while the interaction between the two factors had a significant effect on respiration rate, ascorbic and dehydroascorbic acids, total vitamin C, total phenolic content and antioxidant capacity but no significant difference in the TAC, TSS, pH, TA and TSS/TA ratio. Following, these effects are described in detail.

Effect of wounding intensity on respiration rate of strawberry fruits

Respiration rate gives an immediate overview of the metabolism of a commodity, where higher respiration is an indicator of accelerated metabolism which is inversely related to shelf-life (Kader and Saltveit, 2003). Strawberry is among the commodities with highest respiration rate, which may be further stimulated by wounding. The effect of wounding intensity on the respiration rate of 'Candonga' strawberry fruits after 120 min post-cutting at 5 °C is shown in Figure 1.2. Wounding induced a significant increase in respiration of strawberries tissues that resulted to be proportional to cutting intensity up to a certain point from 10.0 (WHO) to 18.2 ml CO₂ kg⁻¹ h⁻¹ (P64). According to Surjadinata and Cisneros-Zevallos (2003), an increase in respiration may be due to enzyme synthesis and a simultaneous decrease due to the action of an inactivation system that degrades or inactivates the enzyme being formed. Further increase of wounding beyond P128 did not stimulate respiration rate: a significant decrease up to a minimal level of 5.1 mL CO₂ kg⁻¹ h⁻¹ was observed for chopped samples.

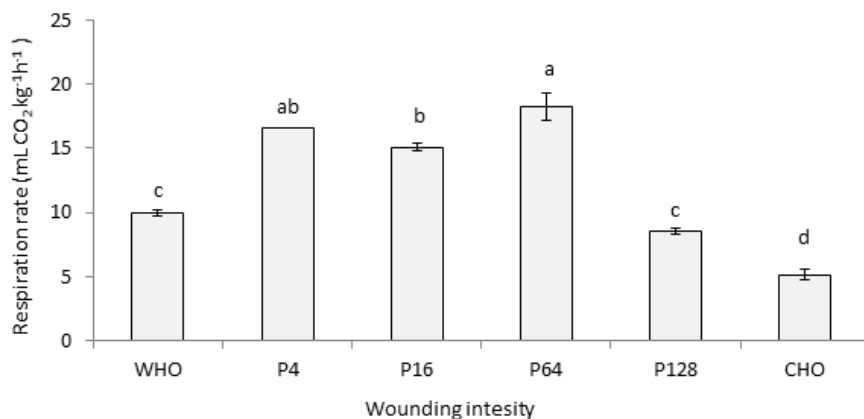


Figure 1.2 Effect of cutting stress on respiration rate at 5 °C of ‘Candonga’ strawberry fruits at 120 min post-cutting; WHO, P4, P16, P64, P128 and CHO representing for whole fruit, cut into 4, 16, 64, 128 pieces and chopped; mean values and standard error (n=3); lowercase letters indicate significant difference among treatment; (P < 0.05) Tukey’s test.

It is possible that, after a certain degree, the damage of the tissue was very high to compromise cell functionality. The transition from respiring to non-respiring tissues after wounding is probably related to the damage of membrane system or mitochondria and consequent disruption of oxidative phosphorylation. Changes in ammonium dihydrogen phosphate (ADP) and ammonium transferase phosphate (ATP) concentrations in wounded tissue indicate that oxidative phosphorylation failed to keep pace with ATP utilization in injured tissue (Lafta and Fugate, 2011). According to Lafta and Fugate (2011), ATP concentrations declined of 41% while ADP increased 31% between day 1 and day 4 after incremental injury of sugar beet root. Costa *et al.* (2011), reported that respiration rates of fresh-cut strawberries was higher than whole fruits and cooling had a

significant influence on respiration rates. This is possibly due to the increased surface exposed to the atmosphere after cutting, allowing oxygen to diffuse into the interior cells more rapidly, and to the increased metabolic activities of injured cells (Saltveit, 1997; Lagarón, 2011; Nilsson and Hedenqvist, 2011). As already mentioned, physical damage such as bruising and surface injury can stimulate respiration, with the degree of increase being proportional to the severity of the damage. A rapid and temporary increase in CO₂ and ethylene production as a response to wounding induced by slicing operation could reduce the shelf life of fresh-cut products (Artés *et al.*, 2007). The presence of moisture in the cut surface may impede gas diffusion, and with the increasing respiration, this could possibly lead to anaerobiosis, causing deterioration of tissue (Saltveit, 1997; Surjadinata and Cisneros-Zevallos, 2003). However, wound-induced respiration depends on the type of tissue, temperature, controlled atmospheres (Watada *et al.*, 1996) and degree of cutting (Zhu *et al.*, 2001). After wounding, respiration rate of carrot tissue showed a typical increase, a maximum peak, and a decrease through time until reaching steady-state respiration values similar to that of carrot tissue before wounding (Surjadinata and Cisneros-Zevallos, 2003). In some plant tissues this may be related to α -oxidation of fatty acids and CO₂, and is responsible for increased respiration after slicing of potato (Gunes and Lee, 1997). According to Surjadinata and Cisneros-Zevallos (2003), wounding triggers important enzymes from the respiration pathway, such as phosphofructokinase and cytochrome oxidase by increasing and decreasing their activity through time, allowing an

increase and decrease in respiration rate. Thus, the greater the tissue damage, the greater the amount of a number of enzymes and substrates reacting together and, therefore, the greater the degree of browning (Gunes and Lee, 1997).

Effect of wounding intensity on nutritional content of strawberry fruits

The effect of wounding intensity on AA, DHA and total vitamin C content (AA+DHA) of fresh-cut ‘Candongga’ strawberries is shown in Figure 1.3. At Day 0, AA and total vitamin C were not significantly affected by the wounding intensity, with almost similar values among treatments ranging from 36.6 (P64) to 44.6 mg 100 g⁻¹ FW (P4). On the other hand, an increase in DHA content could be observed already at Day 0 with a significant difference among treatments ranging between 6.7 mg 100 g⁻¹ FW in the WHO and 21.6 mg 100 g⁻¹ FW in the treatment P128. Like the AA values, the total vitamin C did not vary among the treatments on the processing day, most probably because ascorbic acid represented the larger part of vitamin C. The mean values of total vitamin C content at Day 0 ranged from 50.8 (WHO) to 60.7 (P128 mg 100 g⁻¹ FW). The wounding intensity did not affect the AA and total vitamin C; this was also observed by Costa *et al.* (2011), and may be due to the immediate analysis of samples before the tissue responded to the stress.

After 2 days from cutting a slight increase in AA was observed for P16 and P64 while a noticeable reduction of AA was found increasing cutting stress on the tissue. P128 and CHO samples showed in fact significantly lower values of ascorbic acid (27.4 and 17.2 mg 100 g⁻¹ FW, respectively) than other treatments,

with 64-piece sample showing an intermediate behavior (40.9 mg 100 g⁻¹ FW) (Figure 1.3 A). On the other hand, a significant increase of DHA with the increase of wounding intensity was observed for all samples starting from P16. The lowest and highest DHA level at Day 2 were found with the WHO and CHO samples (11.8 and 54.7 mg 100 g⁻¹ FW) respectively. The highest level of wounding intensity (CHO) induced about 5-fold increase in DHA content (Figure 1.3 B). Usually, enzymatic and non-enzymatic oxidation of AA into DHA results in a significant decrease in ascorbic acid and consequent increase of the DHA. Therefore, after prolonged storage, mechanical and thermal treatments, DHA content can represent a large portion of total vitamin C (Davey *et al.*, 2000; Lee and Kader, 2000). In the present study different behavior were observed: as for P128 and CHO samples the decrease in AA due to oxidation was accompanied by increase in DHA amount ending in a rise of total vitamin C content of the products (Figure 1.3 C). No further oxidation of DHA into 2,3-diketogulonic acid was observed. Regarding P16 and P64 samples at day 2 higher AA amounts were detected, suggesting the occurrence of new synthesis or the presence of different sources of ascorbic acid. Cordenunsi *et al.* (2005) reported that ascorbic acid synthesis in strawberries occurs during the storage period, and temperature may affect it. Cutting stress activated biosynthesis of AA and reduction of AA metabolism, and hence the level of AA remained unchanged in sweet pepper fruits (Imahori *et al.*, 1997). Furthermore, existent literature (Loewus *et al.*, 1958) showed that in detached strawberry fruits, the majority (80%) of D-glucose is

incorporated into L-ascorbic acid by a pathway that does not involve inversion of the carbon skeleton (Valpuesta and Botella, 2004) i.e. the C1 Carbonyl function of L-ascorbic acid is formed by oxidation of C1 of D-glucose). Moreover, Wolucka and Van Montagu (2003) suggested that exogenous L-gulose and L-gulono-1,4-lactone serve as direct precursors of L-ascorbic acid in plant cells and proposed a L-gulose pathway for the *de novo* biosynthesis of vitamin C in plants. As for the present work it was possible to suppose that new synthesized ascorbic acid replaced the oxidized AA. As a result, ascorbic acid content did not decrease over time while a huge increment in DHA occurred for P64 sample. Some authors observed in different strawberry cultivars that DHA evolution during storage, when associated with ascorbic acid retention, can be taken as an evidence of an operative redox system (AA/DHA) acting during cold storage, and there seems to be no fruit-specific pattern of total AA or DHA accumulation, but a cultivar-specific one (Cordenunsi *et al.*, 2005). Moreover, the precursor-product relationships and the identity of several major DHA breakdown products remained unclear. Interesting biological roles in the apoplast for ascorbate's primary oxidation product (DHA) and ascorbate and DHA metabolism in plants is highly significant; however, many questions concerning their catabolic pathways and the identity of major degradation products remain open (Lin and Varner, 1991). DHA is a branch-point in ascorbate catabolism, being either oxidized to oxalate and its esters or hydrolyzed to DKG and downstream carboxypentonates. The oxidation/hydrolysis ratio is governed by reactive

oxygen species status (Parsons *et al.*, 2011). In general, the ratio of DHA/AA may increase during storage although the oxidized form is more prone to decomposition, leading to the loss of biological activity, the changes in AA forms are important in both, technological and nutritional terms (Lee and Kader, 2000; Cordenunsi *et al.*, 2005).

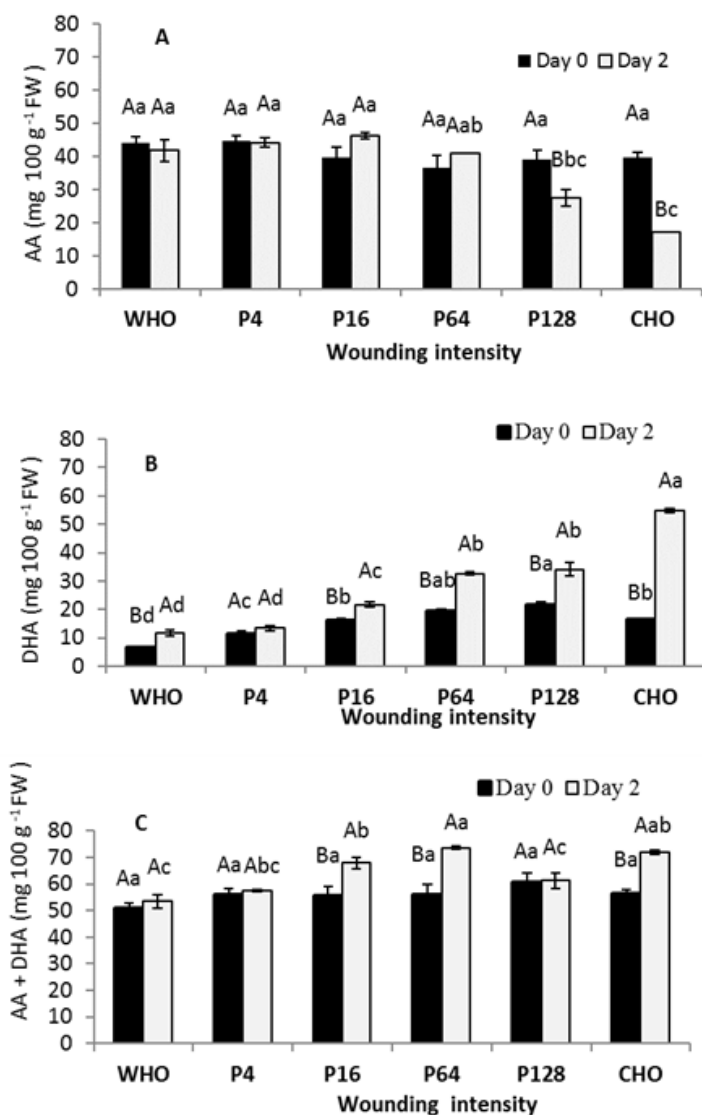


Figure 1.3 Effect of cutting stress on ascorbic acid (AA) (A), dehydroascorbic acid (DHA) (B) and total vitamin C content (C) of fresh-cut ‘Candonga’ strawberries (Day 0 and Day 2 at 5 °C; WHO, P4, P16, P64, P128 and CHO representing for whole fruit, cut into 4, 16, 64, 128 pieces and chopped respectively; mean value and standard error; n=3); different lowercase letters indicate significance levels between treatments and uppercase letters between storage times ($P < 0.05$) Tukey’s test.

Effect of wounding intensity on TPC, TAC, and AC of fresh-cut ‘Candonga’ strawberries is shown in Table 1.1. Wounding intensity significantly affected the TPC of fresh-cut strawberries. At Day 0, P128 had significantly higher ($P < 0.05$) TPC compared to other treatments with the exception of P64, with P16 showing the lowest amount (227.3 mg GAE 100 g⁻¹ FW) and, together with P4 (237.4 mg GAE 100 g⁻¹ FW), resulted in significantly lower content of phenolic compounds than whole fruit. A possible explanation may be found in the prevalence of phenolics *de novo* synthesis with respect to their oxidation. This situation is reversed in the case of a more serious damage. As described by the obtained results, most probably after two days of storage this situation reversed. The damage due to the processing of fresh-cut products increases their antioxidant capacity, inducing synthesis and accumulation of phenolic compounds (Kang and Saltveit, 2002). However, phenolic compounds are also used as precursors for lignin biosynthesis. According to Becerra-Moreno *et al.* (2015), in wounded carrots subjected to additional water stress, lignification processes was favored rather than phenolics biosynthesis (being the phenolic biosynthesis lower than lignification process); thus lower phenolic compounds and higher lignin content was quantified as compared to wounded carrots stored under control conditions. At Day 2 however, TPC increased more clearly in P4 and P16 samples, which showed significantly higher values than other treatments, including the WHO. This is supported by literature reporting that TPC in white potato tubers subjected into different wounding intensities (slices, pie-cuts) showed an accumulation of

100% and 65% higher TPC for slices and pie-cuts, respectively, whereas shredded potatoes stored at 10°C for 96 hours showed 40% lower phenolic content if compared with whole product before storage (Torres-Contreras *et al.*, 2014). Similarly, an accumulation of wounding-induced TPC and an increase of PAL activity in sliced potato tissues compared to whole tubers was found in purple-flesh potato slices stored at 15 °C for 48 hours with approximately 60% increase in TPC (Reyes and Cisneros-Zevallos, 2003). According to a study by Surjadinata and Cisneros-Zevallos (2012), phenolic antioxidant accumulation is dependent on wounding intensity. The phenolic content increased with wounding intensity by 97, 76, and 252% for slices, pieces and shreds, respectively compared to non-wounded carrots (45–52 mg 100 g⁻¹). Phenolic compounds are in fact closely associated with structural and defense-related functions in plants via the phenylpropanoid pathway. After wounding of fruits and vegetables a number of physiological responses are induced, as a result of rupture of the cell membrane, causing the phenolics to combine with the oxidative enzyme systems and another response involves the synthesis of monomeric or polymeric phenolics to repair the wounding damage. Moreover, *de novo* synthesis of PAL, the initial rate-controlling enzyme in phenolic synthesis, is stimulated. This leads to the accumulation of phenolic compounds, and subsequent enzymatic oxidation and tissue discoloration (Adams and Brown, 2007).

There was no significant difference in TAC both at Day 0 and 2 although the mean TAC value of sample P4 showed slight increase during storage time. The

mean value of TAC ranged from 19.1 (P4) to 22.2 (CHO) mg PG-3-glu 100 g⁻¹ FW (Table 1.1). In the literature, there is a wide range of variabilities in the TAC determined. Strawberries TAC has been reported to be in the range between 15.0 to 80.0 mg 100 g⁻¹ FW (Padmanabhan *et al.*, 2016). The increase in anthocyanin concentration during storage may be related to the increase in their biosynthesis and accumulation induced by low temperature storage (Holcroft and Kader, 1999). Accordingly, about 60% accumulation of TAC in sliced potatoes was found by Reyes and Cisneros-Zevallos (2003). Similar increase in anthocyanin was reported in pomegranates stored at low temperatures ([Arendse et al., 2014](#)). Wounding intensity also significantly affected antioxidant capacity of fresh ‘Candonga’ strawberries. At Day 0, samples cut into 16 (635.5 mg TE 100 g⁻¹ FW) and 64 (659.2 mg TE 100 g⁻¹ FW) pieces had significantly higher AC than the CHO (541.0 mg TE 100 g⁻¹ FW) and WHO (519.7 mg TE 100 g⁻¹ FW) samples. A similar trend was also observed after 2 days from cutting when P16 sample showed significantly higher AC value when compared to the CHO samples, although this difference was not significant if compared to the rest of the treatments.

No significant effect of storage time was observed on the TPC, TAC, and AC in CHO sample (Table 1.1), indicating that these compounds were preserved during cold storage. This sample showed also the lowest respiration and no significant difference in total vitamin C if compared to the WHO samples. This characteristic may be exploited to maintain the quality of fresh-blended products. The high

level of vitamin C and phenolics including anthocyanins present in strawberries have antioxidant effects and therefore consequent beneficial effects on the maintenance of consumer health (Giampieri *et al.*, 2012; Van De Velde *et al.*, 2013). The relatively higher values of AC determined for CHO sample could probably be due to the higher values of total vitamin C and phenolics. The AC of fruit is in fact closely correlated to the presence of efficient oxygen radical scavengers, such vitamin C, and phenolic compounds and AC is influenced by individual contribution of different phytochemical compounds (Giampieri *et al.*, 2012). Tulipani *et al.* (2008), demonstrated that vitamin C in strawberries has been found to be the greatest contributor (>30%) of total antioxidant capacity followed by anthocyanins (contributing for 25-40%), and the rest was composed mainly of ellagic acid derivatives and flavonols (Tulipani *et al.*, 2008; Padmanabhan *et al.*, 2016). This can demonstrate that antioxidant activity is indicative of total vitamin C and total phenolic content and therefore anthocyanin and ellagitannins content in strawberries (Giampieri *et al.*, 2012).

TSS, pH value, TA and TSS/TA ratio of fresh-cut strawberry subjected to different wounding intensity and stored for two days at 5 °C were not significantly affected by the interaction between treatments and storage time (data not shown). The mean values of TSS ranged from 7.6 (WHO at Day 0) to 8.6 °Brix for WHO, P16 and CHO samples at Day 2. Almost similar pH values (3.9 to 4.0) were determined for all treatments and sampling times. TA ranging from 0.7 to 0.8 g citric acid 100 g⁻¹ FW showed almost no differences among

treatments and sampling time except for P64 which had a slightly lowest value after two days. Similarly, almost no differences in the TSS/TA ratio were recorded. The highest and lowest values were 9.4 (P4 at day 0) and 12.1 (P16 at Day 2).

Table 1.1 Effect of wounding intensity on TPC (mg GAE 100 g⁻¹ FW), TAC (mg PG-3-glu 100 g⁻¹ FW), and AC (mg TE 100 g⁻¹ FW) of fresh-cut ‘Candonga’ strawberries (Day 0 and Day 2 at 5 °C; WHO, P4, P16, P64, P128 and CHO representing for whole fruit, cutting into 4, 16, 64, 128 pieces and chopped respectively. Mean values ± standard error; n=3); different lowercase letters indicate significance levels between treatments, uppercase letters between storage times (days at 5°C) (P < 0.05), and ns = no significance (P < 0.05) by Tukey's test.

Compound	Days at 5 °C	Wounding intensity					
		WHO	P4	P16	P64	P128	CHO
Total phenolic content	Day 0	288.0 ± 4.2 Ab	237.4 ± 9.1Bc	227.3 ± 2.4 Bc	294.8 ± 1.3 Aab	323.2 ± 5.0 Aa	294.0± 2.7 Ab
	Day 2	261.9 ± 7.1 Bb	318.7 ± 10.1 Aa	324.8 ± 10.1 Aa	252.1 ± 8.8 Bb	287.3 ± 6.0 Bab	258.4 ± 3.8 Ab
Total anthocyanin content	Day 0	19.4 ± 0.3 ns	19.1 ± 0.4 Ans	20.1 ± 0.2 ns	20.9 ± 1.0 ns	20.5 ± 1.1 ns	19.7 ± 0.6 ns
	Day 2	20.7 ± 0.9 ns	20.8 ± 0.1 Bns	21.5 ± 0.8 ns	22.0 ± 0.1 ns	22.0 ± 1.2 ns	22.2 ± 0.7 ns
Antioxidant capacity	Day 0	589.5 ± 2.0 Abc	602.4 ± 2.8 Aabc	635.5 ± 7.6 Aa	659.2 ± 5.4 Aa	627.4 ± 7.3 Aab	541.0 ± 3.8 Ac
	Day 2	519.7 ± 12.5 Bab	597.7 ± 25.7 Bab	616.3 ± 1.3 Ba	544.4 ± 1.6 Bab	566.9± 5.4 Aab	462.4 ± 6.1 Ab

Conclusion

The effect of wounding intensity on respiration rate and compositional values of fresh 'Candonga' strawberry fruits was clearly determined. Wounding intensity, storage time and their interactions had significant influences on respiration rate, AA, DHA and total vitamin C, TPC, and AC. However, these effects proportionally changed up to a certain degree of wounding intensity. The respiration rate related to the wounding intensity increase up to 64 pieces wounding and then decrease when the damage to the cells was very high and compromise their functionality. TAC, TSS, pH, TA and TSS/TA ratio however were not affected by wounding intensity. In general, high degree of wounding intensity (P128 and CHO) caused a significant decrease in respiration rate. In addition, the chopped sample did not show any significant difference in TPC, TAC and AC during storage time.

The results of the present study demonstrated that the application of wounding intensities could be used as simple emerging technology to induce the accumulation of TPC in plants (Torres-Contreras *et al.*, 2014) and selection of appropriate wounding intensity and/or abiotic stress can enhance the nutritional and functional values of fresh produce (Reyes and Cisneros-Zevallos, 2003). These results should be taken into consideration for processing and packaging optimization of minimally processed products from fresh strawberries.

2.2 Influence of cultivar on quality attributes of fresh-blended strawberry purees

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Abstract

BACKGROUND: The objective of this work was to evaluate the influence of cultivar on quality attributes of fresh strawberry purees. The study focused on surveying organoleptic, technological and nutritional attributes of 6 cultivars in order to develop fresh-blended puree based on strawberry fruit. The strawberry cultivars - ‘San Andreas’, ‘Sabrina’, ‘Candongia’, ‘Festival’, ‘Fortuna’ and ‘Nabila’ - were harvested at commercial maturity, transported to laboratory and stored at 5 °C overnight. Freshly blended purees from each cultivar were prepared and physical, chemical and sensory attributes were immediately evaluated. In addition, polyphenol oxidase (PPO) enzyme activity was determined. All data were subjected to one-way analysis of variance and mean values were separated by Tukey’s test ($P<0.05$). Moreover, principal component analysis and correlation methods were also applied.

RESULTS: Significant influence ($P<0.05$) of cultivar was found on colorimetric parameters, viscosity, total soluble solids, pH, titratable acidity, sugars, acids, total phenolic content, total anthocyanin content, antioxidant capacity, and PPO activity. No significant difference on vitamin C content and sensory attributes except color among the studied cultivars. PCA revealed total color difference (ΔE^*) was inversely correlated ($r=76$) ($P<0.001$) with anthocyanin content. Moreover, high correlation between total phenolic content and antioxidant capacity were found. ‘Festival’ followed by ‘San Andreas’ had better sensory color, lower total color difference and higher total anthocyanin content.

Alternatively, ‘Candonga’ had higher values of total phenolic content, total soluble solids, antioxidant capacity and PPO activity.

CONCLUSION: The results suggest that cultivar significantly influences the quality of strawberry purees. Cultivar ‘Festival’ followed by ‘San Andreas’ and ‘Candonga’ can be selected as suitable cultivars for fresh strawberry puree production. Further studies on processing and storage condition optimization would provide better information in the fresh-blended puree processing.

Key words: Anthocyanin, aroma, antioxidant capacity, strawberry, puree

Introduction

Strawberry (*Fragaria x ananassa* Duch.), belonging to the Rosacea family, is a known non-climacteric fruit showing no increase in respiration rate or ethylene production during ripening (Merchante *et al.*, 2013). It is grown in a wide range of climates and, among the berries, its worldwide production ranks second after the grape (Bodelón *et al.*, 2013). Strawberry is a highly demanded fruit by consumers due to its attractive color and flavor but has a very short shelf life (Sulaiman and Silva, 2013). This is mainly due to its susceptibility to mechanical injury, excessive texture softening, physiological disorders and growth of pathogens that can rapidly reduce the quality and make marketing a challenge (Fernández-León *et al.*, 2013; Gol *et al.*, 2013). Strawberries are rich sources of micronutrients and phytochemical compounds including flavonoids such as anthocyanin, flavonols, hydrolysable tannins and phenolic acids (Terefe *et al.*, 2010). Compared with other fruits, strawberries possess high antioxidant activity (Sun *et al.*, 2002). Total antioxidant contents of strawberries were found to be 1.3

times the antioxidant activity of oranges, 2 times that of red grapes, 5 times that of apples and bananas and 13 times that of honeydew melon. Similarly, strawberries have higher total anthocyanin content (from 2 to 11 fold) than apples, peaches, pears, grapes, tomatoes, oranges or kiwifruit (Scalzo *et al.*, 2005). However, these compounds can be influenced by several factors such as genotype, crop conditions (environmental and cultivation techniques), maturity at harvest, and postharvest conditions, including processing (Olsson *et al.*, 2004; Giampieri *et al.*, 2012).

The influence of genotype on quality attributes of strawberries is probably due to the fact that this is the major factor that determine fruit nutritional quality (Sulaiman and Silva, 2013). The concentration of phenolic compounds of 27 strawberry cultivars and their changes during ripening showed significant differences among cultivars. Anthocyanins were the most abundant class of phenolic compounds in the majority of the cultivars, and their amount varied from 8.5 to 65.9 mg 100 g⁻¹ FW contributing by 28% to the total phenolic contents in the strawberry cultivars (Aaby *et al.*, 2012). To this regard some studies focused on influence of environmental and genetic factors on health-related compounds, (Tulipani *et al.*, 2011) confirmed a relevant genotype-dependent response to environmental stress conditions resulting in changes in the antioxidant, phenolic and allergenic properties of strawberry fruits in different years. The anthocyanin composition and color characteristics juices from 39 strawberry genotypes were studied by Bakker *et al.* (1994). They discovered that no single cultivar or

breeding line possessed the innate pigment characteristics thought to confer greater color stability on strawberry juices, but the anthocyanin patterns were far more complex in strawberries. Cultivar screening is an important step in the study of a new product based on fresh fruits, because it allows identifying a cultivar with better responses to postharvest handling and minimal processing (Cabezas-Serrano *et al.*, 2009).

Selection of good quality raw material is vital to the success of minimally processed products. The choice of a cultivar with superior sensorial quality (mainly defined as attractive color and flavor,) and good nutritional quality attributes (high vitamin C anthocyanins content, and antioxidant activity) is essential for consumer's acceptance. Consumers are in fact attracted by the typical aroma and the bright red color of these product (Gössinger *et al.*, 2009). Accordingly, the main factor to be considered choosing the raw material should be the maintenance of fresh-like appearance and organoleptic quality. Moreover, high flow behavior, and long shelf life is particularly important from a technological point of view. According to this direction, research is generally aimed to the selection of strawberry cultivar with the best color stability (expressed as low total color difference (ΔE^*), high total anthocyanin content) and high total phenolic content and antioxidant capacity and low polyphenol oxidase activity, in order to prevent browning.

The main objective of this study was to survey strawberry cultivars grown in Italy for quality and technological attributes of fresh-blended puree in order to evaluate

the extent of variability for each attribute, define differences and common characteristics, and, possibly, identify those which are better suitable to be used as a raw material for fresh- blended puree production.

Materials and methods

Sample preparation

Six strawberry cultivars ('San Andreas', 'Sabrina', 'Candonga', 'Festival', 'Fortuna' and 'Nabila') grown in Basilicata region (South Italy) and harvested at commercial maturity (more than 75% showing red color) (Pelayo-Zaldívar *et al.*, 2005; Jouquand *et al.*, 2008) were screened. After harvest, fruits were transported to the postharvest laboratory (University of Foggia) and stored at 5 °C overnight. Fruits free of physical defects, decay and with uniform color and size were visually selected, washed with tap water and manually dried with paper. After calyx removal, fruits were divided into halves. Subsequently, approximately 500 g of fruit from each cultivar were homogenized using a high power blender (Bimby TM31, Vorwerk, Germany) at a speed of 4000 rpm for 90 s at 5 °C. Immediately after blending, physicochemical, organoleptic and nutritional attributes of puree samples were evaluated or frozen at – 80 °C until analysis. All samples were processed and analyzed in triplicate.

Colour measurements

A spectrophotometer (CM-2600d Konica Minolta, Japan) was used to measure fruit color on the base of 10 fruits per replicate, and of fruit puree sampled on a Petri dish upon blending and after 1 and 7 hours at room temperature (RT),

obtaining L*, a* and b* values. Moreover, Chroma (C*) and hue angle (h°) parameters were calculated using equations 1 and 2, respectively. In addition, total color differences (ΔE*) of purees was calculated after 1 and 7 h after in reference to the initial values (equation 3) as described in Ayala-Zavala *et al.* (2004):

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$h^\circ = \arctan (b^*/a^*) \quad (2)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

According to Ayala-Zavala *et al.* (2004) the total color difference or ΔE* value is the change between the initial and final measure and it is estimated as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0).

Puree viscosity

The viscosity of strawberry puree was determined using a Bostwick Consistometer (43100 Ing.Rossi & Catelli; Parma, Italy). Samples (about 50 g of puree) at 20 °C were placed in the tray closed by a bulkhead with care. After releasing the bulkhead by means of click system, the travel of the sliding of the sample in 30 s was recorded. Final results were expressed in cm * 30 s⁻¹ as reported by Colantuono *et al.* (2015).

Sensory Analysis

Sensory analysis was carried out using a 7-member laboratory panel trained and familiar with the characteristics of the fruit. Panelists were asked to rate puree

samples for color intensity, aroma, sweetness, acidity, homogeneity, off-flavor and overall acceptance scores on a 5-point hedonic scale (1= minimum; 5= maximum) values. The samples were coded with random three-digit numbers in identical plastic cups and order of presentation to each panelist was randomized.

Total soluble solids content pH, titratable acidity

Total soluble solids (TSS) was obtained by measuring the refractive index of strawberry juice using a digital refractometer (Atago RX-7000cx; Atago Co. Ltd., Japan) at 25 °C. Two grams of juice were used for titratable acidity (TA) potentiometric titration (TitroMatic CRISON 1S; Crison Instrument, Barcelona, Spain) against 0.1 N (Sodium hydroxide; NaOH) up to pH 8.20. TA was expressed as g of citric acid equivalent per 100 g fresh weight (g citric acid 100 mL⁻¹ FW). as described in Mahmood *et al.* (2012). The pH value was measured by a calibrated pH-meter (pH 4, pH 7) and TSS: TA ratio was calculated by dividing TSS values by the TA values.

Simultaneous analysis of sugars and organic acids

Sugars and organic acids were extracted by homogenizing 5 g of strawberry purees with 10 mL of ultrapure water. The homogenate was centrifuged at 9000 rpm for 10 minutes at 5 °C. The supernatant was filtered with a C₁₈ Sep-Pak cartridge (Grace PureTM, New York, USA) and with a 0.2 µm filter (INCOFAR, Modena, Italy). All extracts were performed in triplicate samples. Organic acids and sugars were identified using as described by Mena *et al.* (2011). Samples were diluted with ultrapure water (1:1) and were injected (10 µL) into HPLC

system (Agilent 1200 series) equipped with an UV detector, set at 210 nm, coupled with a refractive index detector. Peak separation was achieved on a Rezex ROA-Organic Acid H⁺(8%) column (300 × 7.80 mm) (Phenomenex, Torrance, USA), using a mobile phase of acidified water (phosphoric acid (0.1%) with a flow rate of 0.5 mL min⁻¹ and an oven temperature of 30 °C. The different organic acids and sugars were characterized and quantified by chromatographic comparison with analytical standards. Results were expressed as mg 100 g⁻¹ FW.

Vitamin C, total phenolic, total anthocyanin content and antioxidant capacity evaluation

To measure vitamin C content, 5 grams of strawberry purees were mixed with 10 mL of Methanol- water (5:95; v/v), plus citric acid (21 g L⁻¹), EDTA (0.5 g L⁻¹) NaF (0.168 g L⁻¹). After one minute, the extract was filtered through cheesecloth and the pH was adjusted to 2.2 – 2.4 by addition of 6 mol L⁻¹ HCl. After that the homogenate was centrifuged at 10000 rpm for 5 min. Supernatant was taken and filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then through a 0.2 µm cellulose acetate filter. L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA) contents were determined as reported in Zapata and Dufour (1992) with slight modifications. The HPLC analysis was achieved after derivatization of DHA into the fluorophore 3-(1, 2-dihydroxyethyl) furol [3, 4-b] quinoxaline-1-one (DFQ) with 1, 2-phenylenediamine dihydrochloride (OPDA). Samples of 20 µL were analysed with an HPLC (Agilent Technologies 1200

Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 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 mmol L⁻¹ cetrimide and 50 mmol L potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min⁻¹. The detector wavelengths were 348 nm for DHA and 251 nm for AA. AA and DHA contents were expressed as mg of L-ascorbic or L-dehydroascorbic acid per 100 g of fresh weight (mg 100 g⁻¹ FW).

Total phenolic content (TPC) was analyzed by using the Folin–Ciocalteu as the method reported by Singleton and Rossi (1965). The extract was prepared by adding 10 mL of Methanol: water (80:20 v/v) solution containing 2 mM (84 mg L⁻¹) of Sodium fluoride (NaF) to 5 grams of puree (Singleton and Rossi, 1965a). Extracts were then centrifuged at 5 °C and 9000 rpm for 10 min. 100 µL of extract was mixed with 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L⁻¹). Then samples were left at room temperature for 2 h in dark. The absorbance was read at 725 nm against a blank (using a spectrometer (UV-1700 Shimadzu, Jiangsu, China). TPC was calculated based on the calibration curve of gallic acid and results were expressed as milligram gallic acid equivalents per 100 g fresh weight (mg GAE 100 g⁻¹ FW).

For total anthocyanin content (TAC), one gram of strawberry puree was mixed with 20 mL of extraction solution (methanol in 1% hydrochloric acid; HCl-

MeOH mixture) and homogenized for 20 s using Ultraturrax (IKA T18 basic, Wilmington, NC, USA). The homogenate was then centrifuged for at 4 °C at 2000 rpm, 15 min. Then 700 µL of supernatant plus 300 µL of 1% HCl-MeOH solution was put in cuvettes and total anthocyanin was read immediately in spectrophotometer at 510 nm as described by Cordenunsi *et al.* (2002). Results were expressed mg of pelargonidin-3-glucoside equivalents per 100 g of fresh weight (mg PG-3 glu 100 g⁻¹ FW).

Antioxidant capacity assay (AC) was performed according to Brand-Williams *et al.* (1995), using the same extract obtained for TPC. A sample of 50 µL was pipetted into 950 µL of DPPH (2, 2-Diphenylpicrylhydrazyl) solution and absorbance was read after 24 h at 515 nm. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was used as a standard and results were expressed in milligrams of Trolox equivalents per 100 g of fresh weight (mg TE 100 g⁻¹ FW).

Polyphenol oxidase (PPO) activity

The extraction and assay of PPO was carried according to Terefe *et al.* (2010), with modifications. Five grams of strawberry samples and 10 mL of ice-cold extraction solution (0.2 M sodium phosphate buffer (pH=6.5) consisting 4% (w/v) polyvinylpolypyrrolidone (PVPP) were homogenized for 1 min. After centrifugation at 12,000 ×g for 30 min at 4 °C, the supernatant was used as the crude enzyme extract for the PPO assay. The reaction mixture contained 0.5 ml of extract, and 1.5 ml of a substrate solution 0.07 M catechol (Sigma-Aldrich,

Italy) as the substrate added to 0.05 M of a sodium phosphate buffer (pH=6.5) solution. The blank was prepared in the same way except that 0.2 M phosphate buffer (pH=6.5) was used instead of the crude enzyme extract. The absorbance of the assay mixture was monitored at 400 nm at 25 °C for 2 min using a UV–visible spectrophotometer (UV-1700 Pharma spec, Shimadzu, Japan) in a kinetic mode according to Terefe *et al.* (2010). The activity of the enzyme was expressed as the change of absorbance per min per gram of fresh weight of sample ($\text{nmol min}^{-1} \text{g}^{-1} \text{FW}$).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and significant differences between means were separated by Tukey's test at $P < 0.05$ (95% confidence level). Moreover, multivariate analyses such as principal component analysis (PCA) and linear correlation coefficients (r) between certain pairs of parameters were also applied to measure and further explain the data variability. Biplot technique was used to display the relative positioning of quality attributes of the first two PCs. All statistical analyses were performed with Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA USA) software.

Results and discussion

Influence of cultivar on strawberry purees quality attributes

Color parameters

Color is one of the most important quality attribute of strawberries and colour retention during processing or storage is a determinant parameter to be considered

in fresh strawberry puree production. The instrumentally evaluated colour attributes of whole strawberry fruits of 6 cultivars and fresh-blended purees obtained from them are reported in Tables 2.1 and 2.2. The colour attributes of fruit surface varied significantly among the cultivars. The average L^* values ranged from 34.4 ('Festival') to 38.9 ('Nabila'). Significantly lighter red fruit surface (higher L^* values) were observed in 'Nabila' and 'Candongga' fruits compared to other cultivars. This was partly expressed by the highest a^* value of 'Nabila', 'Sabrina' and 'Candongga' compared with 'Fortuna', 'San Andreas' and 'Festival'. Significantly higher b^* values were recorded for 'Nabila' and 'Candongga' strawberries compared to the other cultivars although the b^* values in 'Candongga' and 'Sabrina' were not significantly different. Regarding to the Chroma (C^*), 'Nabila', 'Sabrina' and 'Candongga' fruits showed significantly higher C^* values compared to the others. Cultivars 'Festival' and 'San Andreas', which had among the lowest L^* , a^* , b^* and Chroma values, also showed the highest hue angle (h°) values compared to other cultivars (Table 2.1). The significant variability in the colour parameters can be attributed to several factors including maturity stage, genotype and cultivar (Wang and Camp, 2000).

For puree production, colour attributes just after blending is also important in order to select suitable raw material. Table 2.2 shows the initial colour attributes of the 6 fresh-blended purees. Also in this case L^* value varied significantly among cultivars. 'Sabrina' had the lightest colour compared to other cultivars

except 'Candonga' which was also not different from 'Nabila'. The average L* values ranged from 37.6 in 'Fortuna' to 44.5 in 'Sabrina'. 'Fortuna' had significantly higher L* values compared to 'Festival' and 'San Andreas' which presented a darker colour. The a* values (redness) of purees varied from 20.4 in 'Nabila' to 30.2 in 'San Andreas'. However, the a* values of 'San Andreas', 'Sabrina' and 'Candonga' were not statistically different among each other although these values were significantly higher than in 'Nabila' puree which had the lowest a* value. There was no significant difference among the a* values of fresh-blended purees from 'Nabila', 'Fortuna' and 'Festival'. The purees yellowness expressed as b* value varied from the highest in 'Candonga' and 'Sabrina' (10.6 and 9.0) to the lowest in 'Nabila' and 'Festival' (3.8). These values however, were not statistically different comparing with the b* values of 'San Andreas' and 'Fortuna'. The Chroma values of the purees were significantly different among the cultivars. 'Candonga', 'San Andreas', and 'Sabrina' had significantly higher values compared to 'Nabila' which was not different from 'Fortuna' and 'Festival'. Also Hue angle (h°) values were found to differ significantly from the highest (81.7) in 'Festival' and 'Nabila' (80.0) to the lowest in 'Candonga'. Moreover 'Fortuna' was found to be different from 'Candonga' but not with the others (Table 2).

The color of whole fruits were not clearly correspondent to the respective fresh-blended purees. It was observed that the surface and inner part of fruits did not have the same colour intensity, giving them a different appearance after blending.

A more red fruits may possibly have less red flesh color or *vice versa*. In addition, the presence of achenes (seeds) (number, color and morphology) on the surface may influence peel color, and then this effect could be not observed in the purees when the seeds are dispersed. According to Cordenunsi *et al.* (2002), the strongly colored inner parts of ‘Mezi’ strawberries give them a more homogeneous appearance. In contrast, in ‘Campineiro’ fruits anthocyanins were predominantly distributed on the surface, resulting in fruits slightly coloured red in the surface and pink to white in the inner parts.

In spite of the variability among cultivars, color retention of the product during a given storage time is a valuable parameter in order to evaluate cultivar(s) suitability for puree production. Total colour differences (ΔE^*) would provide further information on how far these color characteristics are maintained during short-time handling. Therefore, the ΔE^* values of the fresh-blended purees obtained from the 6 cultivars at 1 and 7 h after blending at room temperature were calculated and they are reported in Figure 2.1. Just 1 h after blending, the ΔE^* was already well visible ($\Delta E^* > 3.0$) in all cultivars except ‘Festival’ which was just slightly noticeable with a ΔE^* equal to 1.8. Total color variation of ‘Festival’ followed by ‘San Andreas’ were instead not noticeable. After 7 h at RT, this total color variation was well-visible in the purees from ‘Candonga’, ‘Fortuna’ and ‘Nabila’ while for ‘San Andreas’ and ‘Festival’ was still slightly noticeable. Therefore, low total color difference, is one of most desirable characteristics of fresh purees that indicates the relative better color retention. As already

mentioned this quality attribute was clearly seen in ‘Festival’ (Fig.1) and can be an important criterion for selecting it as suitable cultivar for puree production.

Table 2.1 Color attributes of whole strawberry fruits of six cultivars. Mean values, n=10; values in the same row with different letters are significantly different (P<0.05) by Tukey’s test.

Attribute	Strawberry cultivar					
	San Andreas	Sabrina	Candonga	Festival	Fortuna	Nabila
L*	35.0 b	34.7 b	37.6 a	34.4 b	35.7 b	38.9 a
a*	28.5 b	31.5 ab	31.1 ab	24.0 c	29.3 b	32.2 a
b*	5.3 de	7.7 bc	8.9 ab	4.4 e	6.4 cd	10.4 a
Chroma (C*)	29.1 c	32.5 ab	32.5ab	24.5 d	30.1 bc	33.9 a
Hue angle (h°)	79.7 a	76.5 c	74.4 d	80.3 a	78.1 b	72.5 e

Table 2.2 Initial color attributes and Bostwick viscosity of freshly blended purees obtained from six strawberry cultivars. Mean values, n=3; values in the same row with different letters are significantly different (P < 0.05) by Tukey's test.

Attribute	Strawberry cultivar					
	San Andreas	Sabrina	Candonga	Festival	Fortuna	Nabila
L*	34.0 d	44.5 a	43.1 ab	32.2 d	37.6 c	41.3 b
a*	30.2 a	27.6 a	28.8 a	25.9 ab	25.5 ab	20.4 b
b*	8.0 ab	9.0 a	10.6 a	3.8 b	6.3 ab	3.8 b
Chroma (C*)	31.2 a	29.1 a	30.7 a	26.2 ab	26.4 ab	20.8 b
Hue angle (h°)	75.1 abc	72.4 bc	69.7 c	81.7 a	77.2 ab	80.0 a
Viscosity (cm *30 s⁻¹)	8.0 a	8.0 a	6.8 b	7.7 ab	6.7 c	7.7 ab

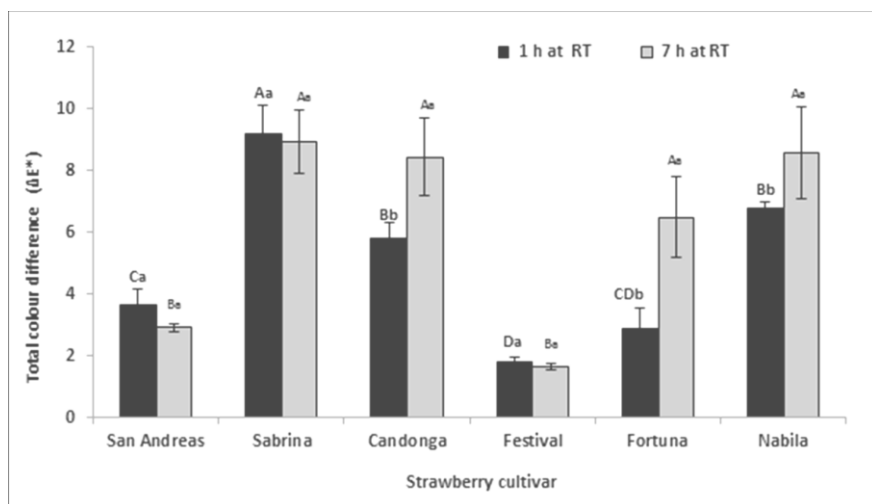


Figure 2.1 Total color difference (ΔE^*) of purees obtained from six strawberry cultivars after 1 and 7 h of storage at room temperature; mean values and standard deviation ($n=3$). Different uppercase letters indicate statistically significant differences among cultivars and lowercase letters between storage time ($P<0.05$) Tukey's test.

Summarizing, results of color parameters showed that 'Festival' had the highest hue angle, both as a fruit and as a fresh-blended puree, with a low value of L^* and low saturation, which indicated a paler shade of red, compared to other cultivars. In addition, the lowest ΔE^* values in 'Festival' revealed a better color retention compared among the cultivars.

'Festival' puree showed in fact the lowest total color variation both after 1 and after 7 hours and the highest content of anthocyanins (as described further below). The higher TAC in this cultivar could be significantly related to the less noticeable color changes during the first 7 h at room temperature. Moreover, the slighter color variation during time was also confirmed by the sensory results where color intensity of 'Festival' puree was significantly higher. The higher

anthocyanin content indicates a greater color intensity of the product, that will make any change of color less apparent (Bursac Kovacevic *et al.*, 2015). Ahmed *et al.* (2004) reported a similar linear relationship between color and anthocyanin contents in plum puree. Similarly, Cao *et al.* (2012) found that ΔE^* values showed a negative correlation with anthocyanin and ascorbic acid contents in cloudy and clear strawberry juices. Therefore, the stability of both anthocyanins and ascorbic acid may influence the total color difference.

The viscosity of fresh strawberry purees as measured by Bostwick consistometer is shown in Table 2.1 The flow of fresh strawberry purees were found to range from 6.7 to 8.0 cm* 30 s⁻¹. The lowest and highest values were observed in ‘Fortuna’ and ‘San Andreas’ and ‘Sabrina’, respectively. Viscosity for ‘Candongra’ resulted to be significantly lower than ‘San Andreas’ and ‘Sabrina’. These results showed significant fluctuation among the cultivars. Viscosity varies depending on cultivar, maturity at the time of processing, heat, presence of enzymes like pectin methyl esterase (PME) and polygalacturonase (PG) in the raw material (Tiziani and Vodovotz, 2005; Aguiló-Aguayo *et al.*, 2009). In general, this parameter can be affected by TSS, pectin and apparatus characteristics during storage. Both chemical and enzyme-catalyzed reactions can occur during storage and modify the pectin characteristics affecting therefore viscosity (Bermejo-Prada *et al.*, 2015). As result, the viscosity decay can occur quickly. Consequently, preservation of viscosity at more or less similar values of the freshly blended product is important.

Influence of cultivar on sensory and chemical quality attributes

Sensory and chemical attributes mainly affect quality of fruits and vegetables-based products. Results of sensory evaluation of fresh strawberry purees are shown in Table 2.3. As also seen from instrumental determination, colour variation within cultivars was also appreciated by sensorial scores. The colour intensity score of puree from 'Fortuna' strawberries was significantly lower than for other cultivars while the highest values were perceived in purees from 'Festival' and 'San Andreas'. No significant differences were perceived for other considered sensorial attributes. Panelists score for colour, aroma, homogeneity, acidity and overall acceptance for all the studied cultivars were higher than 3 (limit of usability). Sweetness scores were lower than 3, with 'Festival' slightly sweeter than others although these differences were not significant, due to the high variability of the value among the panelists. The overall acceptance scores of purees ranged from good to excellent. Despite the low sweetness all samples were rated with high values (>4). Not only the good color of the purees influenced the high acceptance rates but may be the good balances of sweetness and acidity. Nonetheless, many of these sensory parameters can be influenced by the chemical composition.

Table 2.3 Sensorial attributes of fresh strawberry purees of six cultivars: n=21 (7 panelists and 3 replicates); scale 1= minimum and 5 =maximum score values. Mean values in the same rows with different lowercase letters are significantly different ($P < 0.05$) by Tukey's test; ns = no significance.

Sensory attributes	Strawberry cultivar					
	San Andreas	Sabrina	Candong	Festiva	Fortuna	Nabila
Color intensity	4.7 a	3.9 ab	3.9 ab	4.9 a	3.7 b	4.2 ab
Aroma	3.9 ns	4.3 ns	3.9 ns	4.4 ns	4.1 ns	4.4 ns
Sweetness	2.1 ns	2.3 ns	2.1 ns	2.6 ns	2.4 ns	2.4 ns
Acidity	3.3 ns	3.4 ns	3.7 ns	3.4 ns	3.2 ns	3.6 ns
Homogeneity	2.9 ns	3.3 ns	3.6 ns	3.8 ns	3.6 ns	3.4 ns
Off-flavor	1.3 ns	1.0 ns	1.0 ns	1.0 ns	1.0 ns	1.1 ns
Overall acceptance	5.0 ns	4.5 ns	4.6 ns	4.9 ns	4.4 ns	4.7 ns

Table 2.4 Chemical attributes of fresh strawberry purees from six cultivars. Mean values in the same rows with different lowercase letters are significantly different ($P < 0.05$) by Tukey's test; ns = no significance).

Chemical Attribute	Strawberry cultivar					
	San Andreas	Sabrina	Candonga	Festival	Fortuna	Nabila
Total soluble solids (TSS) (°Brix)	7.8 b	9.4 a	9.1 a	8.1 b	7.6 b	8.0 b
pH value	3.5 b	3.6 b	3.7 b	3.9 a	3.8 ab	3.7 b
Titrateable acidity (TA) (g citric acid 100 mL ⁻¹ juice)	0.8 a	0.6 b	0.8 a	0.7 ab	0.4 c	0.5 c
TSS/TA ratio	9.8 c	15.7 ab	11.4 bc	11.6 bc	19.0 a	16.0 ab
Sucrose (mg 100 g ⁻¹ FW)	1168.1 a	1205.7 a	1025.1 ab	763.1 b	900.7 ab	1080.9 a
Glucose (mg 100 g ⁻¹ FW)	1316.4 ns	1390.7 ns	1509.1 ns	1392.7 ns	1184.6 ns	1520.6 ns
Fructose (mg 100 g ⁻¹ FW)	170.1 ab	200.6 a	187.4 ab	192.8 ab	161.9 b	190.3 ab
Citric Acid (mg 100 g ⁻¹ FW)	302.2 ab	229.2 b	418.5 a	344.3 ab	381.3 a	285.8 ab
Tartaric acid (mg 100 g ⁻¹ FW)	18.1 ns	13.7 ns	20.3 ns	20.7 ns	18.5 ns	19.9 ns
Malic acid (mg 100 g ⁻¹ FW)	225.9 ns	227.6 ns	273.2 ns	258.5 ns	240.7 ns	263.9 ns
Succinic acid (mg 100 g ⁻¹ FW)	13.6 b	11.2 b	22.4 ab	36.7 ab	48.8 a	35.7 ab
Fumaric acid (mg 100 g ⁻¹ FW)	0.2 c	0.5 abc	0.5 abc	0.7 a	0.3 bc	0.6 ab
Ascorbic acid (mg 100 g ⁻¹ FW)	23.6 ns	22.4 ns	23.2 ns	20.6 ns	18.6 ns	21.8 ns
Dehydroascorbic acid (mg 100 g ⁻¹ FW)	2.8 ns	2.4 ns	3.4 ns	2.7 ns	1.9 ns	2.8 ns
Total Vitamin C (mg 100 g ⁻¹ FW)	26.3 ns	24.8 ns	26.5 ns	23.3 ns	20.5 ns	24.6 ns
Total phenolic content (mg GEA 100 g ⁻¹ FW)	161.8 b	130.4 b	190.6 a	163.3 b	130.1 b	147.1 ab
Total anthocyanin content (mg PG-3-glu 100 g ⁻¹ FW)	38.0 b	33.2 b	25.0 c	45.4 a	24.4 c	16.6 c
Antioxidant capacity (mg TE 100 g ⁻¹ FW)	301.2 b	290.3 b	395.5 a	324.4 ab	289.0 b	299.9 b

The results of the chemical analysis on fresh strawberry purees are shown in Table 2.4. Significant variability of chemical attributes was observed on most of the parameters.

Total soluble solids, titratable acidity and the ratio of TSS/TA are important factors for evaluating the quality of strawberry cultivars (Kafkas *et al.*, 2007). The TSS content of ‘Sabrina’ and ‘Candonga’ were significantly higher than in other cultivars. Mean values ranged from 7.6 to 9.4 °Brix in ‘Fortuna’ and ‘Sabrina’, respectively. The pH of the purees did not present high variability, ranging from 3.5 for ‘San Andreas’ to 3.9 for ‘Festival’, the latter presenting the highest value significantly different from all other varieties with the exception of ‘Fortuna’. Despite the low values of pH, titratable acidity (TA) resulted very low for all cultivars ranging from 0.4 to 0.8, with ‘Candonga’ presenting almost 2 times the TA if compared with ‘Fortuna’. This was also confirmed by sensorial analysis that showed a difference, although not statistically significant, between the acidity of these cultivars (3.7 and 3.2 for ‘Candonga’ and ‘Fortuna’, respectively). On the contrary, sensory results about sweetness were not supported by instrumental data, with ‘Festival’ showing the highest value and ‘Sabrina and Candonga’ the lowest. However, also in this case, no significant differences were observed in panelists evaluation. The relationship between pH and TA did not result very consistent, with cultivars presenting the highest pH value also presenting among the highest TA (*i.e.* of cultivar ‘Festival’). The maturity index or index of palatability (expressed by the TSS/ TA ratio), may

further explain the relationship of TSS and TA contents. The lowest and highest ratios were found to be 19.0 ('Fortuna') and 9.8 ('San Andreas'). Also organic acids were instrumentally evaluated, main organic acids in strawberry-based purees were citric and malic, while tartaric, succinic, ascorbic, and fumaric were contained in lower amounts. Purees from 'Candonga' followed by 'Fortuna' were found to be significantly higher in citric acid than that from 'Sabrina', the latter showing the lowest content. Tartaric and malic acids were not statistically different among cultivars while succinic acid was significantly higher in 'Fortuna' (48.8 mg 100 g⁻¹ FW) compared to 'San Andreas' and 'Sabrina' (13.6 and 11.2 mg 100 g⁻¹ FW, respectively), the latter presenting less than one fourth the content of 'Fortuna'. Also content in fumaric acid was highly variable within the cultivars with 'Festival' showing more than 3 times the content found in 'San Andreas' (Table 2.4). As can be observed cultivar 'Fortuna' showed high content of organic acids, and simultaneously the lowest levels of vitamin C but also had the highest TSS/TA ratio. This may well explain the lowest sensory acidity if compared with the other cultivars, showing therefore a more balanced flavor. In fact, ripe strawberry flavor is expressed by the right sugar-acid balances (Pineli *et al.*, 2011), but the accumulation of soluble sugars and organic acids and is strongly dependent on genotypes (Kafkas *et al.*, 2007).

Glucose, fructose and sucrose are by far the most abundant soluble components in strawberries (Gündüz and Özdemir, 2014). As it is shown in Table 2.4, sucrose and fructose were found to significantly differ among cultivars. The highest

sucrose content was found in ‘San Andreas’, ‘Sabrina’ and ‘Nabila’ (1205.7, 1168.1 and 1080.9 mg 100 g⁻¹ FW, respectively) if compared with ‘Festival’ (763.1), while ‘Candongá’ (1025.1 mg 100 g⁻¹ FW) was in the middle. Glucose content was relatively higher than sucrose for all cultivars although differences within cultivars were not significant. Fructose content in ‘Sabrina’ (200.6 mg 100 g⁻¹ FW) was significantly higher than in ‘Fortuna’ (161.9 mg 100 g⁻¹ FW), while the other cultivars content was in between, and did not show significant differences. This information also confirmed the results concerning total soluble solids: for this parameter in fact ‘Sabrina’ and ‘Candongá’ showed the highest content, while ‘Fortuna’ puree had lower soluble solids content (Table 2.4), so it is possible to state that high TSS values were mainly due to a high sugars content (especially sucrose and fructose).

Vitamin C, total phenolic, total anthocyanin content and antioxidant capacity

Strawberry, together with other berries, represents one of the most important sources of bioactive compounds with antioxidant activity, being the richest sources of natural antioxidants among fruit (Cordenunsi *et al.*, 2002). Genetic background plays an important role on the chemical antioxidant profile of strawberries as reported by Tulipani *et al.* (2008). The antioxidant properties of strawberry fruits are related to the high content of L-ascorbic acid (vitamin C), anthocyanins and phenolic compounds (Erkan *et al.*, 2008; Pineli *et al.*, 2011; Álvarez-Fernández *et al.*, 2014), which have been medically recognized as having positive influences on protecting against the risk of many diseases (Zhu

et al., 2013; Giampieri *et al.*, 2014). As Table 4 indicates, the ascorbic acid, dehydroascorbic acid and total vitamin C content (AA +DHA) were not significantly different among the studied cultivars. The average total vitamin C content was about 23.7 mg 100 g⁻¹ FW with AA content of 21.0 mg 100 g⁻¹ FW and DHA content of 2.7 mg 100 g⁻¹ FW. The highest and lowest values of total vitamin C were found in ‘Candonga’ and ‘Fortuna’ respectively although this difference was not statistically significant. These findings were in accordance with the recent report by Šamec *et al.* (2016) that the average total vitamin C value was not significantly different among cultivars. In general there is a high variability in vitamin C content among strawberry cultivars from Italy ranging from 23.0 to 47.0 mg 100 g⁻¹ FW (Tulipani *et al.*, 2008). On the other hand, Cordenunsi *et al.* (2002); Tulipani *et al.* (2008) reported a strong variability in vitamin C content among nine strawberry genotypes, a 2-fold difference was found. This could be mainly attributed to the sample preparation and storage. According to Cordenunsi *et al.* (2005), vitamin C content at harvest time and its changes during storage seem to be dependent on cultivar, besides cultural practices, light intensity, and climatic conditions.

Strawberries are also a good source of phenolic compounds, of which anthocyanins are the most abundant fraction that are responsible for the red colour of strawberries fruit (Aaby *et al.*, 2012)..

Total anthocyanin content (TAC) was highly variable and significantly different ($P<0.05$) among the studied cultivars. The highest and lowest TAC were in

'Festival' and in 'Nabila' with 45.4 and 16.6 mg PG-3-glu 100 g⁻¹ FW, respectively. With 'San Andreas' and 'Sabrina' showing an intermediate pattern (Table 2.4). Cordenunsi *et al.* (2002) also reported that TAC ranged greatly among cultivars, from 13.0 mg 100 g⁻¹ FW in 'Campineiro' to 55.0 in 'Mazi'. Anthocyanin content is affected by many factors such as strawberry cultivar, postharvest performance, cultivation practice and processing (Cordenunsi *et al.*, 2002; Fernandes *et al.*, 2012; Pincemail *et al.*, 2012). Major anthocyanin content detected in 'Festival' partially explained its observed lowest total color variation of this sample.

The phenolic content of strawberries is highly variable mainly due to cultivar differences (Pineli *et al.*, 2011; Aaby *et al.*, 2012; Vandendriessche *et al.*, 2013). In the present study, there was a significant variability ($P < 0.05$) among the cultivars. Total phenolic content (TPC) in 'Candonga' was significantly higher than in all other cultivars except 'Nabila'. The TPC of the studied cultivars ranged from 130.1 in 'Fortuna' to 190.6 mg GAE 100 g⁻¹ FW in 'Candonga' (Table 2.4). As shown in Table 2.4, also antioxidant capacity (AC) was significantly different ($P < 0.05$) among the studied cultivars. The highest AC was found in 'Candonga' which was significantly different from all other cultivars, with the exception of 'Festival', which showed an intermediate behaviour. As will be described in the last section of the present work, TPC resulted to be highly related to AC, the first strongly contributing to the antioxidant properties of the product.

PPO activity

The PPO activity of fresh strawberry purees from the six studied cultivars is shown in Figure 2.2. It was relatively low and significantly variable ($P < 0.05$) among cultivars. The highest PPO activity was found in ‘Candongga’ with $2.0 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ FW}$ while the lowest was found in ‘Nabila’ with $1.0 \text{ nmol min}^{-1} \text{ g}^{-1} \text{ FW}$. The PPO activity of ‘Candongga’ was significantly higher than all cultivars with exception of ‘Sabrina’. Moreover, no statistically significant difference in PPO activities were observed among the purees from ‘Festival’, ‘San Andreas’, ‘Fortuna’ and ‘Nabila’. Because there is a direct relationship between enzymatic activity and color changes, the lower the activity the higher the color stability, which was observed in ‘Festival’. This cultivar showed very low PPO activity and, as expected, the lowest ΔE^* . This result was similar to the values reported by Terefe *et al.* (2009) and sometimes can be as low as $0.1 \text{ (nmol min}^{-1} \text{ FW)}$ (Terefe *et al.*, 2013) for ‘Festival’ strawberry purees.

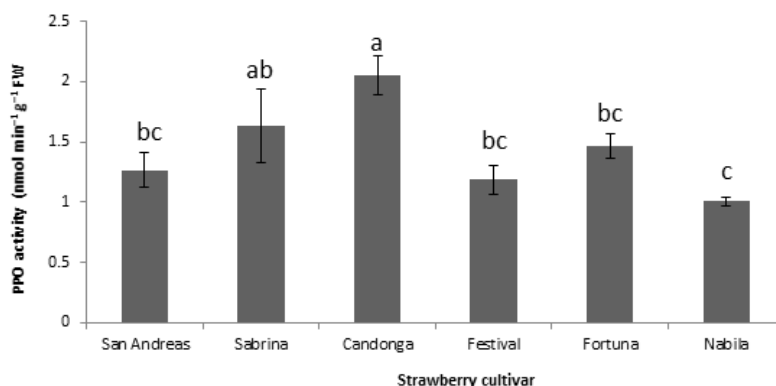


Figure 2.2 PPO activity of fresh strawberry purees from six cultivars; mean value (n= 3) error bars=standard deviation. Different letters indicate significant difference among cultivars

Principal component analysis and correlations

Principal component analysis (PCA) is a widely used multivariate analytical statistical technique to explain differentiation between samples and to obtain more information on the variables that mainly influence the sample similarities and differences (Šamec *et al.*, 2016). This procedure extracts the dominant patterns in the data matrix in terms of a complementary set of loading plots (Rodríguez-Delgado *et al.*, 2002). PCA searches a linear combination of the studied variables in order to maximize the total variance explained. If the variables are highly correlated, they will be combined into a component that will explain the highest quantity of variance in the sample (observations) (Pineli *et al.*, 2011).

Figure 2.3 indicates the PCA biplot related to quality attributes of freshly blended strawberry purees from six cultivars. The biplot demonstrates the relative position of the six considered cultivars between them and in comparison with the variables. Factors loading values are the correlations of each variable with the PC representing as vectors (positions) in the space resulted by the axes of the biplot graphic. For each cultivar the three points on the biplot represented a replicate. In the graphic, variables and cultivars that are close to each other, and in the same geometric plan of the biplot, are interrelated, and they are instead distant from variables and observations to which they are not related, or even negatively related. According to Pineli *et al.* (2011), the greater is the distances from the origin of the axis, the greater is the correlation of the variable with the PC represented in that dimension. Differences and common properties among the studied cultivars in relation to the variables can be presented. The first principal component (PC1) and the second (PC2) describe 36.4% and 23.7%, respectively and both explained approximately 60.1% of total data variability (Figure 2.3). TAC and ΔE^* are positively and negatively associated to PC1 respectively. ‘Festival’ followed by ‘San Andreas’, located at the positive top end of this PC, resulted to be correlated with high TAC and low ΔE^* showing therefore better color retention. TPC, TA, AA, DHA, and AC and ‘Candongá’ followed by ‘San Andreas’ were correlated to positive right hand of PC1. In addition, TSS and PPO activity, and ‘Candongá’ and to some extent ‘Sabrina’ were correlated to the positive end of PC1 indicating that these cultivars were found to be related with

high values of these variables. Based on these positions, 'Festival', 'San Andreas' and Candonga' were related to most of the preferable quantities of these quality variables. On the other hand, 'Nabila 'and 'Sabrina', being closer to the origin, were related to less preferable variables. 'Fortuna' was associated to the PC2 and not correlated to most of the preferable characteristics (Figure 3). Results of a linear correlation of some variables done (data not shown) showed significant correlation among the variables. There was a strong correlation of TPC with AC ($r=0.80$; $P<0.001$), considerable correlation with DHA ($r=0.56$; $P<0.05$) and TA ($r=0.51$; $P<0.05$) but weak correlation with TAC ($r=0.37$; $P>0.05$). In addition, there were moderate correlation between TA and AA ($r=0.65$; $P<0.05$); while AC was also moderately correlated to DHA ($r=0.48$; $P<0.05$). The ΔE^* was inversely correlated to the TAC ($r=-0.76$; $P\leq 0.001$).

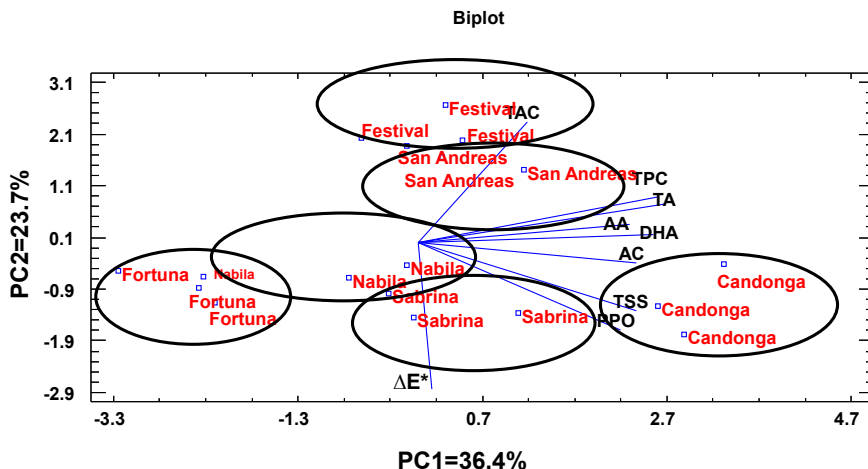


Figure 2.3 PCA biplot on certain quality attributes of freshly blended strawberry purees from six cultivars; (ΔE^* =Total color difference, TSS= Total soluble solids, TA= Titratable acidity, TPC= Total phenolic content, AC= Antioxidant capacity (DPPH), TAC= Total anthocyanin content, AA= Ascorbic acid, DHA=Dehydroascorbic acid and PPO= Polyphenol oxidase).

In the present study, although no significant correlation was found between AA and TPC or AC, the DHA was moderately correlated with both TPC and AC which may indicate that DHA also contributed to the high antioxidant capacity of the fresh-blended purees. A positive correlation between antioxidant activity and total phenols and vitamin C was reported by (Cornacchia *et al.*, 2011) approving both contribute to the AC in potato samples. The strong correlation found between TPC, and AC can be attributed to the fact that both assays rely on the same reaction mechanism. Pineli *et al.* (2011) found higher values of TPC in ‘Osogrande’(290.9 mg GAE 100 g⁻¹ FW) and ‘Camino Real’(174.4 mg GAE 100 g⁻¹ FW) strawberry cultivars at green, pink and ripe stages. The authors stated

that considerable positive correlation of TPC and anthocyanin content (66%) was observed among the cultivars. Pineli *et al.* (2011) reported a considerable positive correlation between TPC and AC. Weak correlation between TPC and AC was reported by Meyers *et al.* (2003). Although the potential interaction between phytochemicals was complex to explain, the weak or lack of correlations between TPC and AC could be partially related to the use of not well-characterized cultivars. It would be of interest to further explore in more detail the intercultural differences in specific major and minor phenolic compounds (Meyers *et al.*, 2003). According to Cordenunsi *et al.* (2002), TAC is important not only for color or attractiveness but also as benefits to the consumer's health contributing to the AC.

In this work, although TAC was not significantly correlated to TPC, considerable positive correlation of TAC and AC was found. However, according to the findings by Meyers *et al.* (2003), anthocyanin was not correlated to antioxidant capacity even though the antioxidant properties of strawberry fruits are related to the high content of L-ascorbic acid (vitamin C), anthocyanins and phenolic compounds are reported by (Erkan *et al.*, 2008; Pineli *et al.*, 2011).

Conclusion

Several factors can influence the nutritional and physicochemical composition of strawberries including genotype, maturity stage, harvest season, planting location, climatic factors, and plant management. Among these, cultivar is a major factor in determining fruit quality and suitability for a certain use (Sulaiman and Silva, 2013; Livinali *et al.*, 2014).

The present study investigated into the physical, chemical, sensory attributes and enzyme activity of six strawberry cultivars in order to possibly identify those which are better suitable to be used as a raw material for freshly blended puree production. Results revealed significant differences in most of the evaluated attributes although only few of them showed prominent variability providing further important information on the physical, chemical and sensory attributes of fresh strawberry purees.

Color, viscosity, TSS, TA, pH and TSS: TA ratio, TPC, AC, TAC, fructose, sucrose, citric, succinic, fumaric acids and PPO activity were significantly variable among cultivars. Most of the sensory quality attributes except the color intensity were not significantly different among the cultivars. Moreover, total vitamin C (AA+DHA) of fresh-blended strawberry purees was not different among the cultivars.

According to these results, for fresh-blended strawberry puree production, 'Festival' followed by 'San Andreas' which had better color stability (lower ΔE^* , higher TAC) and 'Candongga' which had higher TPC and AC may be selected as among the suitable cultivars for this purpose. Further studies on processing and storage condition optimization are essential for quality puree production.

2.3 Effects of blending time and temperature on quality attributes of fresh strawberry purees

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Abstract

Optimum blending condition plays an important role in the quality of fresh-blended purees. The effects of blending time and temperature on quality attributes of fresh strawberry puree were optimized in two independent trials - response surface method based on a central composite design (Trial I) and blending temperature (Trial II) experiments. For Trial I, ten different blending time/temperature experimental runs ranging from 5 to 140 s and from 0 to 21°C respectively were tested. For this purpose, fresh ‘Festival’ strawberries grown in Valenzano (BA) (South Italy) were harvested and transported to the Postharvest lab (University of Foggia) and held at 0, 3, 10, 18 and 21°C until they reached temperature. Fruits were blended using high power blender at 4000 rpm according to the designed runs. Physicochemical, organoleptic and nutritional quality attributes were evaluated as responses at the processing day (Day 0) and after 6 days at 5°C (Day 6). All data were fitted to the second-order (quadratic) regression equation. Analysis of variance was applied to determine significant differences. Standardized Pareto charts and estimated surface responses were used to identify the impact of variables on responses and optimize various

responses. Results showed that at day 0, blending time significantly affected L* and b* values, titratable acidity and succinic acid, whereas the temperature had significant influence on total anthocyanin content, sucrose, fructose, and malic acid. After 6 days, however, viscosity, total anthocyanin content, and dehydroascorbic acid were the only parameters found to be significantly affected by the blending conditions. Viscosity was affected by combined effects of blending time and temperature but not by the individual factors. High blending temperature (room temperature, 21°C) positively influenced the total anthocyanin content but had a negative effect on dehydroascorbic acid. The applied blending conditions did not have significant effects on the sensory attributes. Blending temperature was relevant in Trial I. Trial II was therefore aimed to better understand the effect of this factor on selected quality parameters during storage of fresh purees. Strawberries held at 5 or 21°C until they reached temperature were blended at 4000 rpm for 90 s and stored for 13 days at 5°C. About 250 g of purees were stored in glass bottles (250 ml) at 5°C. Color parameters (L*, a*, b* and ΔE^*), vitamin C, total anthocyanin content and sensorial attributes were monitored after 0, 3, 8 and 13 days at 5°C. All data of Trial II were subjected to a two-way analysis of variance and Tukey's test ($P < 0.05$) was used to separate the means. After 3 days, samples blended at the lowest temperature showed significantly better color maintenance (lower ΔE^*), aroma and overall acceptance. Also ascorbic acid content was stable. As for the highest blending temperature only total anthocyanins were benefited probably

due to the ease of extraction. In general, blending time less than 90 s and temperature below 10°C had no significant impact on several evaluated parameters and, specifically, 5 °C process was better than room temperature (21°C). Quality of the obtained purees were maintained for more than 8 days. Packaging condition improvement using different plastic films under active and or passive MAP should be considered for better quality preservation.

Key words: Anthocyanin, aroma, central composite design, ‘Festival’, smoothies

Introduction

Strawberry (*Fragaria x ananassa* Duch) is a known non-climacteric fruit with an attractive color, flavor and aroma and a frequent consumption worldwide. Strawberry is a relevant source of bioactive compounds due to its high level of vitamin C and phenolics (Van De Velde et al., 2013). This high levels of bioactive compounds are essential for the nutritional and organoleptic qualities of fresh and minimally-processed products based on strawberry (Tulipani *et al.*, 2008). The latter type of products has met increasing favor by consumers mainly because of the ease-of-use and lack of preparation time, and because of the high, fresh-like quality, which are main characteristics of these products. Among these, fresh-squeezed or fresh-blended smoothies and purees are becoming more and more popular. In order to obtain good quality strawberry-based purees, the conventional thermal processing may not be suitable. Impactful treatments on soft fruits, such as berries can result in adverse loss of organoleptic and nutritional quality of the product. The color and flavor of strawberries significantly

deteriorate during thermal processing (Patras et al., 2009; Terefe et al., 2010). According to Patras et al. (2009), strawberry purees treated by conventional thermal processes showed an overall decrease of 22.6% in ascorbic acid content compared to the high pressure processed samples. Anthocyanin pigments also readily degrade during thermal processing with dramatic impact on the characteristic color of fruits. High temperature treatments impact on key quality parameters influencing consumer sensory acceptance and may also affect nutritional properties (Patras *et al.*, 2010). This has led to the development of alternative non-thermal processing technologies to produce foods with a minimum of nutritional, physicochemical, or organoleptic changes induced by the technologies themselves (Oms-Oliu *et al.*, 2012; Ubeda *et al.*, 2014).

The consumers demand for products that retain their original nutritional values, with minimum negative effects of processing, more natural, produced in an environmental-friendly way, with high quality, additive free, and with an extended shelf life. The current trend is rapidly shifting towards freshly squeezed/blended fruit juices and ready-to-eat or drink added-value products. Optimum blending condition remains to be an important need for such beneficial products. This work was aimed to evaluate the effects of blending time and temperature on quality attributes of fresh strawberry puree and determine optimal blending conditions for quality attributes of fresh strawberry puree from nutritional, organoleptic and technological points of view.

Materials and Methods

TRIAL I

Sample preparation and puree processing

‘Festival’ strawberries (*Fragaria x ananassa* Duch.) were harvested at commercial maturity (about 75% showing red color) from Valenzano (BA) (Apulia Region; South Italy); transported to postharvest laboratory (University of Foggia). Fruit were divided into 4 groups (approximately 4 kg each) and held at 0, 3, 10, 18, and 21°C in order to reach the process temperature. Fruit were washed with tap water and dried on a paper towel. After calyx removal, fruit were divided into halves; subsequently, approximately 500 g of fruit were homogenized using a high power blender (Bimby TM31, Vorwerk, Germany) at a speed of 4000rpm at different blending conditions (blending time and temperature combinations). Immediately after blending (Day 0) and after 6 days at 5°C (Day 6), physicochemical, organoleptic and nutritional attributes of puree samples were evaluated. Each treatment was considered as single run.

Experimental design

A response surface methodology (RSM) based on a central composite design (CCD) was applied to optimize blending conditions (time and temperature) of fresh strawberry purees. RSM allows understanding the interaction among factors as well as optimizing the factors that may influence the process outcomes (Baş and Boyacı, 2007; Bezerra et al., 2008). It includes four major steps: experimental design, model fitting, model validation and condition optimization (Khuri, 2006; Dubrovi et al., 2011).

Whereas, CCD optimizes the process, and reduces cost and time required for experimentation by decreasing the number of experiments to be performed in laboratory (Kiran et al., 2016). CCD consists of three types of points: cube points that come from factorial design, axial points and center points. The range and central point values of two independent variables i.e. blending time and temperature are shown in Table 3.2. A factorial design (2^2 + star) approach consisting of 10 experimental runs was employed (Table 3.3). Moreover, the methodology requires that experiments outside the experimental range previously defined should be performed to allow the prediction of the response functions outside the cubic domain (denoted as $\pm \alpha = 1.414$; Table 3.2) as reported by Torrades and García-Montaño (2014). Second-order polynomial equation was used to express the dependent variables as a function of the independent variables. The response functions (y) were partitioned in linear, quadratic, and interaction components, and the data were fitted to the second-order (quadratic) regression equation (1).

$$Y = \beta_0 \sum_{i=1}^2 \beta_i X_i + \sum_{i=1}^2 \beta_{ii} X_i^2 + \sum_{i \neq j=1}^2 \beta_{ij} X_i X_j \quad (1)$$

In this equation, Greek letters betas (β) are the coefficients determined experimentally, Y is the dependent variable and refers to the predicted response, β_0 is the intercept; β_i , β_{ii} , and β_{ij} are the linear, quadratic and interaction coefficients of the model respectively, X_i and X_j are the independent variables, and i and j are the indices of the factors. The operating variables were considered at three levels, namely low (-1), central (0) and high (1).

Table 3.2 Independent values and their levels used for central composite design for fresh-blend Festival’ strawberry puree of two factors (blending time and temperature; X_i and X_j are the independent variables and i and j the indices of the factors also represented by A and B respectively).

Independent variables	Coded				
	-1.414	-1	0	+1	+1.414
X_i : A: Blending time (s)	5	25	72	120	140
X_j : B: Temperature ($^{\circ}$ C)	0	3	10	18	21

Table 3.3 CCD experimental runs as resulted by the combination of 5 level for each of the 2 experimental factors (Blending time (s) and temperature ($^{\circ}$ C) for fresh-blended ‘Festival’ strawberry puree of blending time and temperature factors.

Experimental No.	Coded		Variables	
	X_i	X_j	A: Blending time (s)	B: Temperature ($^{\circ}$ C)
1	0	- 1.414	72.5	-0.1
2	+1	-1	120.0	3.0
3	-1	-1	25.0	3.0
4	- 1.414	0	5.3	10.5
5	0	0	72.5	10.5
6	0	0	72.5	10.5
7	+1.41 4	0	139.7	10.5
8	-1	+1	25.0	18.0
9	+1	+1	120.0	18.0
10	0	+1.41 4	72.5	21.1

Color and viscosity measurements

A spectrophotometer (CM-2600d Konica Minolta, Japan) was used to measure fruit puree color of the puree sampled on a Petri dish upon blending and after 6 days of storage at 5°C, obtaining L*, a* and b* values. Moreover, Chroma (C*) and hue angle (h°) parameters were calculated using equations 2 and 3, respectively as described in Ayala-Zavala *et al.* (2004):

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (2)$$

$$h^\circ = \arctan (b^*/a^*) \quad (3)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (4)$$

The viscosity of puree was determined using a Bostwick Consistometer (43100 Ing. Rossi & Catelli; Parma, Italy). Samples (about 50 g of puree) at 20°C were placed in the tray closed by a bulkhead with care. After releasing the bulkhead by means of click system, the travel of the sliding of the sample in 30s was recorded. Final results were expressed in cm * 30 s⁻¹ as reported by Colantuono *et al.* (2015).

Sensory Analysis

Sensory analysis was carried out using a 7-member laboratory panel trained and familiar with the characteristics of the fruit. Panelists were asked to rate puree samples for color intensity, aroma, sweetness, acidity, homogeneity, off-flavor and overall acceptance scores on a 5-point hedonic scale (1= minimum; 5= maximum) values. The samples were coded with random three-digit numbers in identical plastic cups and order of presentation to each panelist was randomized.

Total soluble solids content, pH, titratable acidity

Total soluble solids (TSS) was obtained by measuring the refractive index of strawberry juice using a digital refractometer (Atago RX-7000cx; Atago Co. Ltd., Japan) at 25°C. Two grams of juice were used for titratable acidity (TA) potentiometric titration (TitroMatic CRISON 1S; Crison Instrument, Barcelona, Spain) against 0.1 N (Sodium hydroxide; NaOH) up to pH 8.20. TA was expressed as g of citric acid equivalent per 100 g fresh weight (g citric acid 100 mL⁻¹ FW). as described in Mahmood *et al.* (2012). The pH value was measured by a calibrated pH-meter (pH 4, pH 7) and TSS/ TA ratio was calculated by dividing TSS values by the TA values.

Simultaneous analysis of sugars and organic acids

Sugars and organic acids were extracted by homogenizing 5 g of strawberry purees with 10 mL of ultrapure water. The homogenate was centrifuged at 9000 rpm for 10 minutes at 5°C. The supernatant was filtered with a C₁₈ Sep-Pak cartridge (Grace Pure TM, New York, USA) and with a 0.2 µm filter (INCOFAR, Modena, Italy). All extracts were performed in triplicate samples. Organic acids and sugars were identified as per method described by Mena *et al.* (2011). Samples were diluted with ultrapure water (1:1) and were injected (10 µL) into HPLC system (Agilent 1200 series) equipped with an UV detector, set at 210 nm, coupled with a refractive index detector. Peak separation was achieved on a Rezex ROA-Organic Acid H+(8%) column (300 × 7.80 mm) (Phenomenex, Torrance, USA), using a mobile phase of acidified water (phosphoric acid (0.1%)

with a flow rate of 0.5 mL min^{-1} and an oven temperature of 30°C . The different organic acids and sugars were characterized and quantified by chromatographic comparison with analytical standards. Results were expressed as $\text{mg } 100 \text{ g}^{-1}$ FW.

Vitamin C, total phenolic, total anthocyanin content and antioxidant capacity evaluation

To measure vitamin C content, 5 grams of strawberry purees were mixed with 10 mL of Methanol- water (5:95; v/v), plus citric acid (21 g L^{-1}), EDTA (0.5 g L^{-1}) NaF (0.168 g L^{-1}). After one minute, the extract was filtered through cheesecloth and the pH was adjusted to 2.2 – 2.4 by addition of 6 mol L^{-1} HCl. After that the homogenate was centrifuged at 10000 rpm for 5 min. Supernatant was taken and filtered through a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA) and then through a $0.2 \text{ }\mu\text{m}$ cellulose acetate filter. L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA) contents were determined as reported in Zapata and Dufour (1992) with slight modifications. The HPLC analysis was achieved after derivatization of DHA into the fluorophore 3-(1, 2-dihydroxyethyl) furol [3, 4-b] quinoxaline-1-one (DFQ) with 1, 2-phenylenediamine dihydrochloride (OPDA). Samples of $20 \text{ }\mu\text{L}$ were analyzed with an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column ($150 \text{ mm} \times 4.6 \text{ mm}$; $5 \text{ }\mu\text{m}$ particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/ H_2O (5: 95 v/v) containing

5 mmol L⁻¹ cetrimide and 50 mmol L potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min⁻¹. The detector wavelengths were 348 nm for DHA and 251 nm for AA. AA and DHA contents were expressed as mg of L-ascorbic or L-dehydroascorbic acid per 100 g of fresh weight (mg 100 g⁻¹ FW).

Total phenolic content (TPC) was analyzed by using the Folin–Ciocalteu as the method reported by Singleton and Rossi (1965). The extract was prepared by adding 10 mL of Methanol: water (80:20 v/v) solution containing 2 mM (84 mg L⁻¹) of sodium fluoride (NaF) to 5 grams of puree (Singleton and Rossi, 1965a). Extracts were then centrifuged at 5°C and 9000 rpm for 10 min. 100 µL of extract was mixed with 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L⁻¹). Then samples were left at room temperature for 2h in dark. The absorbance was read at 725 nm against a blank (using a spectrometer (UV-1700 Shimadzu, Jiangsu, China). TPC was calculated based on the calibration curve of gallic acid and results were expressed as milligram gallic acid equivalents per 100 g fresh weight (mg GAE 100 g⁻¹ FW).

For total anthocyanin content (TAC), one gram of strawberry puree was mixed with 20 mL of extraction solution (methanol in 1% hydrochloric acid; HCl-MeOH mixture) and homogenized for 20s using Ultraturrax (IKA T18 basic, Wilmington, NC, USA). The homogenate was then centrifuged for at 4°C at 2000 rpm, 15 min. Then 700 µL of supernatant plus 300 µL of 1% HCl-MeOH solution was put in cuvettes and total anthocyanin was read immediately in

spectrophotometer at 510 nm as described by Cordenunsi *et al.* (2002). Results were expressed mg of pelargonidin-3-glucoside equivalents per 100 g of fresh weight (mg PG-3 glu 100 g⁻¹ FW).

The antioxidant capacity (AC) assay was performed according to Brand-Williams *et al.* (1995), using the same extract obtained for TPC. A sample of 50 μ L was pipetted into 950 μ L of DPPH (2, 2-Diphenylpicrylhydrazyl) solution and absorbance was read after 24 h at 515 nm. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was used as a standard and results were expressed in milligrams of Trolox equivalents per 100 g of fresh weight (mg TE 100 g⁻¹ FW).

Polyphenol oxidase (PPO) activity

The extraction and assay of PPO was carried according to Terefe *et al.* (2010), with modifications. Five grams of strawberry samples and 10 mL of ice-cold extraction solution (0.2 M sodium phosphate buffer (pH=6.5) consisting 4% (w/v) polyvinylpolypyrrolidone (PVPP) were homogenized for 1 min. After centrifugation at 12,000 \times g for 30 min at 4°C, the supernatant was used as the crude enzyme extract for the PPO assay. The reaction mixture contained 0.5 ml of extract, and 1.5 ml of a substrate solution 0.07 M catechol (Sigma-Aldrich, Italy) as the substrate added to 0.05 M of a sodium phosphate buffer (pH=6.5) solution. The blank was prepared in the same way except that 0.2 M phosphate buffer (pH=6.5) was used instead of the crude enzyme extract. The absorbance

of the assay mixture was monitored at 400 nm at 25°C for 2 min using a UV–visible spectrophotometer (UV-1700 Pharma spec, Shimadzu, Japan) in a kinetic mode according to Terefe *et al.* (2010). The activity of the enzyme was expressed as the change of absorbance per min per gram of fresh weight of sample ($\text{nmol min}^{-1} \text{g}^{-1} \text{FW}$).

Statistical analysis

All data were fitted to the second-order regression model using RSM based on CCD. The significance of the model coefficients was determined by analysis of variance (ANOVA). Standard Pareto charts and estimated response surfaces were generated to identify and determine the effect of the independent variables on the various responses. The vertical line in the Pareto charts indicates statistical significance at the 95% confidence level. Using Stat Graphics Centurion XVI (Stat Point Technologies, Inc., Warrenton, VA USA) software, surface response plots were generated in order to optimize the various responses and analyze their behavior in relation to changes in variables. To determine the regression model for each response, empirical model coefficients and R^2 values were calculated to fit models to real data.

TRIAL II

Sample preparation

‘Festival’ strawberries were divided into two different groups (approximately 3 kg each) and stored overnight at 5 and 21 °C, representing cold storage and room temperature, respectively. Subsequently, approximately 1 kg of fruit for each replicate were homogenized at 4000 rpm for 90 s at 5 or 21 °C using a blender (VORWERK Folletto-Bimby TM31, Italy). Color parameters (L^* , a^* , b^*), vitamin C, total anthocyanin content and sensorial attributes were evaluated at 0, 3, 8 and 13 days at 5 °C. All samples were prepared and analyzed in triplicates. Color evaluation, nutritional quality assessment and sensory analysis were carried out as previously reported for Trial I.

Statistical analysis

Two-way ANOVA with treatment and storage time factors and their interactions were run to analyze the data. Significant means were separated by the Tukey’s HSD test ($P < 0.05$) using the Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA USA) software.

Results and Discussion

TRIAL I

Blending time and temperature are important factors affecting the physicochemical, nutritional and sensorial attributes of fresh fruit purees (Colantuono et al., 2015). Optimization of blending factors may be therefore vital in order to produce acceptable quality attributes. In the present study, the effects

of blending time and temperature on quality attributes of fresh-blended 'Festival' strawberry puree were monitored using RSM based on CCD. Results of the present study showed that among the several evaluated attributes, only a few were significantly influenced by the blending conditions. For many of the response variables, the blending conditions did not significantly affect ($P > 0.05$) quality attributes, with exception of color parameters (L^* and b^* values), TA, sucrose, fructose, malic and succinic acids at day 0, and viscosity, TAC and DHA after 6 days of storage at 5°C (Day 6). The coefficients of multiple determinations (R^2 and R^2 -adjusted) for product quality parameters immediately after blending (Day 0) are shown in Table 3.4 whereas Table 3.5 reports R^2 and R^2 -adjusted for significantly affected dependent variables after 6 days of storage. P -value ≤ 0.05 indicates that the model adequately fitted the experimental data. On the other side, P -value > 0.05 means that blending factors did not affect the response variables (data not shown). Therefore, standardized Pareto charts and estimated surface response plots were generated only for the significant response variables.

Table 3.4 Effect of blending time (s) and Temperature (°C) on some quality attributes of fresh-blended ‘Festival’ strawberry purees at processing day (Day 0); * ($P \leq 0.05$); ** ($P \leq 0.01$) = significant difference; ns = no significance (Tukey’s test).

Effect	L*	b*	Titratable acidity	Total anthocyanin content	Sucrose	Fructose	Malic acid	Succinic acid
A: Blending Time (s)	*	*	ns	ns	ns	*	ns	ns
B: Temperature (°C)	ns	ns	*	ns	**	ns	*	*
AA	ns	ns	ns	ns	ns	ns	ns	ns
AB	ns	ns	ns	ns	ns	ns	ns	ns
BB	ns	ns	ns	*	ns	ns	ns	ns
P-value	0.0326	0.049	0.0322	0.019	0.008	0.0495	0.0239	0.0316
R ²	76.3%	71.6%	82.8%	82.6%	87.2%	84.0%	90.4%	88.9%
R ² adjusted	46.6%	36.0%	61.3%	60.9%	71.1%	57.3%	74.5%	70.4%

Table 3.5 Effect of blending time (s) and Temperature (°C) on viscosity, total anthocyanin and dehydroascorbic acid of fresh-blended ‘Festival’ strawberry purees after 6 days at 5 °C (Day 6); * ($P \leq 0.05$) = significant difference; ns = no significant (Tukey’s test).

Effect	Viscosity	Total anthocyanin content	Dehydroascorbic acid
A: Blending Time (s)	ns	ns	ns
B: Temperature (°C)	ns	ns	*
AA	*	ns	ns
AB	ns	ns	ns
BB	*	*	ns
P-value	0.0264	0.0199	0.0264
R ²	82.3%	81.3%	81.7%
R ² adjusted	60.2%	57.8%	58.7%

Effect of blending time and temperature on color and viscosity

The effect of blending time and temperature on the initial L^* and b^* values of fresh-blended strawberry purees are presented in Figure 3.1. Graphically, the standard Pareto charts and estimated surface response plots (Figure 3.1 A and B) indicate that blending time had significant negative effects on color parameters. With the increase of blending time a drop in the values of L^* and b^* was observed. Therefore, strawberry fruits should be blended for less than 140 s at 17 °C in order to maintain higher L^* values (38.3). As for b^* values, it was observed that the lowest blending time (5 s) combined with the higher temperature (21 °C) gave a higher b^* value (8.8). The blending conditions applied in the current experiment had no significant effects on a^* , Chroma and hue angle values at both Day 0 and 6. For red strawberry products both the increase and decrease of lightness can possibly imply color deterioration, indicating either tissues fading or browning (Wang et al., 2015). Since a^* value and hue angle were not different among the treatments, the change in L^* or b^* may not be associated with changes in red color of the puree. A brilliant red color is associated with “freshness” and “healthiness” as opposed to dark red, which may lead to rejection of the product (Cordenunsi et al., 2003). Similarly, high blending time (≥ 80 s) of fresh melon purees significantly affected color parameters (b^* and Chroma) (Colantuono et al., 2015). At Day 6, however, color attributes of fresh-blended strawberry puree were not different among the treatments.

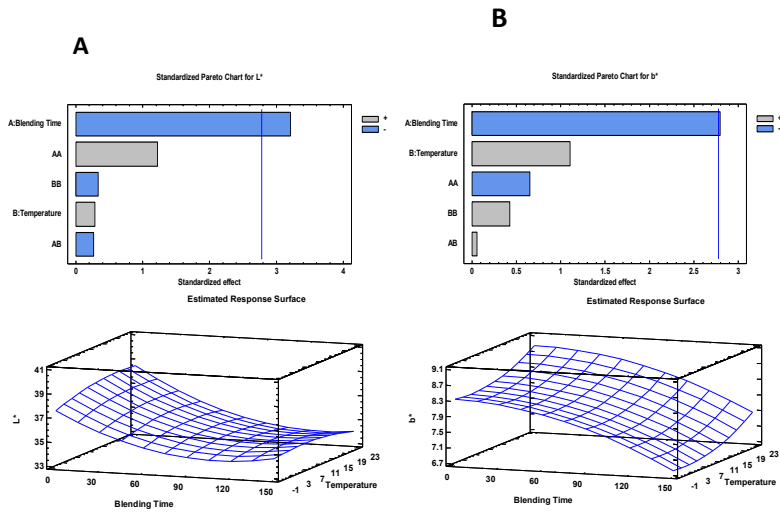


Figure 3.1 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on (A) L* and (B) b* values of fresh-blended ‘Festival’ strawberry puree at Day 0.

Viscosity is an important quality attribute limiting the consumer acceptability of fruit juices. The viscosity of fresh-blended strawberry purees in all used conditions was not significantly different immediately after blending (Day 0). After 6 days at 5°C, however, the quadratic effects of both blending time (AA) and temperature (BB) had significant inversely proportional effects on viscosity (Table 3.4). The standardized Pareto chart and estimated surface response are reported in Figure 3.1 and indicate that increasing the blending factors the viscosity increased significantly first and then decreased. For acceptable quality purees, viscosity should basically not change from the initial values. In order to preserve high viscosity ($13.3 \text{ cm}^2 \text{ s}^{-1}$) at day 6, strawberries need to be blended for not more than 83 s and temperature should be less than 10°C.

According to Maceiras et al. (2007) the processing temperature significantly affected the apparent viscosity of strawberry purees. However also sugar content of strawberry may have an influence on the viscosity of the final product. For higher processing temperature, the observed decrease in viscosity may be due to the inactivation of some enzymes such as pectin methyl esterase (PME) and polygalacturonase (PG) during treatment (Aguiló-Aguayo et al., 2009). Significant linear and combined effects of blending time and temperature on the consistency of melon purees was also reported by Colantuono et al. (2015). However, viscosity varies depending on cultivar, maturity at the time of processing, heat and presence of PME and PG in the raw material (Tiziani and Vodovotz, 2005; Aguiló-Aguayo et al., 2009).

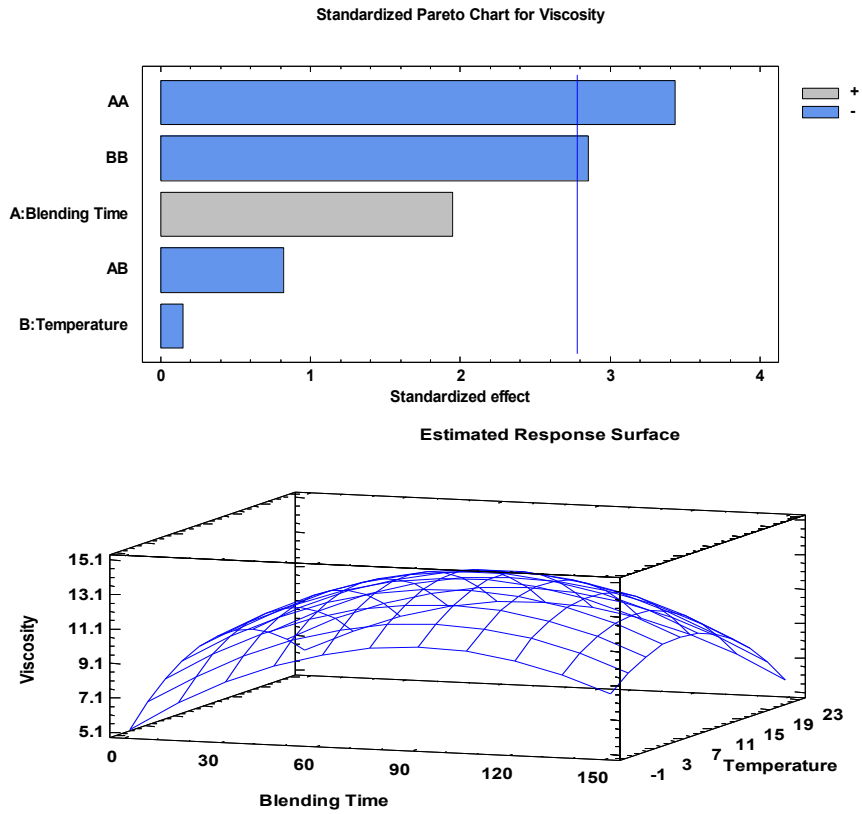


Figure 3.2 Standardized Pareto chart (top) and estimated surface response (bottom) of blending time (s) and temperature ($^{\circ}\text{C}$) factors on viscosity of fresh-blended ‘Festival’ strawberry puree at Day 6.

Effects of blending time and temperature on chemical attributes

Blending conditions significantly affected the titratable acidity (TA) at Day 0. Temperature negatively affected TA: when fruits were blended at higher temperatures the TA value tends to decrease (Figure 3.3). The maximum TA value (0.8 g citric acid 100 g⁻¹ juice) was obtained when strawberry fruits were blended for short time (5 s) at temperature not exceeding 8°C. The decrease in TA at Day 0 can be therefore related to the storage temperature before blending. The proportion of organic acids in fruits noticeably changes according to the storage temperature (Marsh et al., 2004). Although the flavor of strawberry depends on sugar/acid balance, TA is one of the factor that gives different perception of acidity in sensorial evaluation of fruit and fruit-based products. Therefore, the composition of sugars and organic acids, as well as sugar/acid balance, may have an influence on taste (Nishiyama et al., 2008). In the present study, TSS, pH, TSS/TA ratio as well as all sensorial attributes were not different among treatments although blending conditions showed significant influence on sucrose, fructose, malic and succinic acids at Day 0.

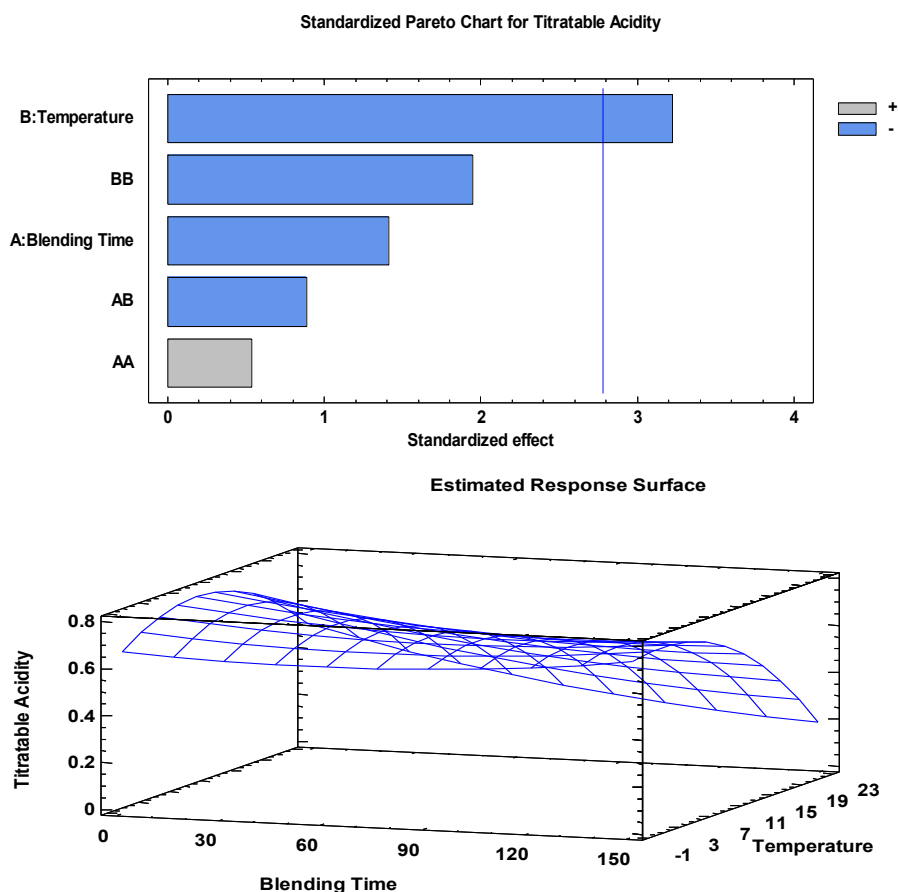


Figure 3.3 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on TA (g citric acid 100 mL⁻¹ juice) of fresh-blended ‘Festival’ strawberry purees at Day 0.

The effect of blending time and temperature on sucrose and fructose at day 0 is presented in Figure 3.4 (A and B). Blending temperature negatively affected the content of sucrose. Blending time had instead a slightly positive effect on the fructose content at Day 0 as can be observed in Figure 3.4 B. The higher the blending time, the higher the fructose content. The blending conditions applied in the present study did not have any significant effects on the glucose content of

the strawberry purees. After 6 days of storage however, no differences in sugar content were observed for all treatments. When strawberries were blended for 140 s at 21°C, the sucrose content decreased while fructose increased. In this regards, probably sucrose was converted into fructose when processed at the highest levels of blending conditions (140 s and 21°C). Because sucrose is the primary source of glucose and fructose, the increased amount of these monosaccharides could account for the decrease in sucrose level (Cordenunsi et al., 2005).

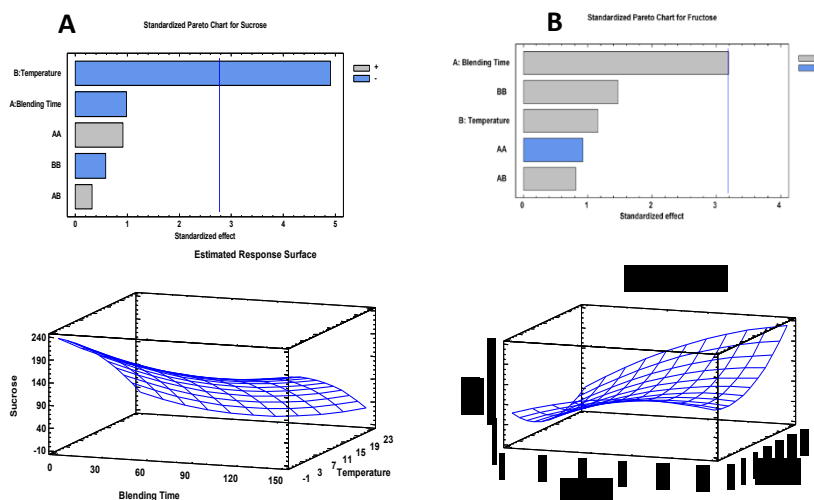


Figure 3.4 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on (A) sucrose (B) fructose (mg 100 g⁻¹ FW) of fresh-blended ‘Festival’ strawberry puree at Day 0.

The blending condition significantly affected the malic and succinic acids of the purees (Figure 3.5). Blending temperature had a significant negative effect on the malic acid content. The standardized Pareto chart and estimated surface response

of malic acid reported in Figure 3.5 A indicate the noticeable effects of processing factors on malic acid. Instead, blending temperature had a positive effect on the content of succinic acid. Figure 3.5 B demonstrates that increasing blending temperature, the succinic acid also increased. The highest blending time and temperature (blending for 140 s at 21°C) resulted in an increase in the succinic acid content that probably is related to carbohydrates fermentation due the processing. Short blending time (5 s) at 10°C did not have negative effect on malic acid content.

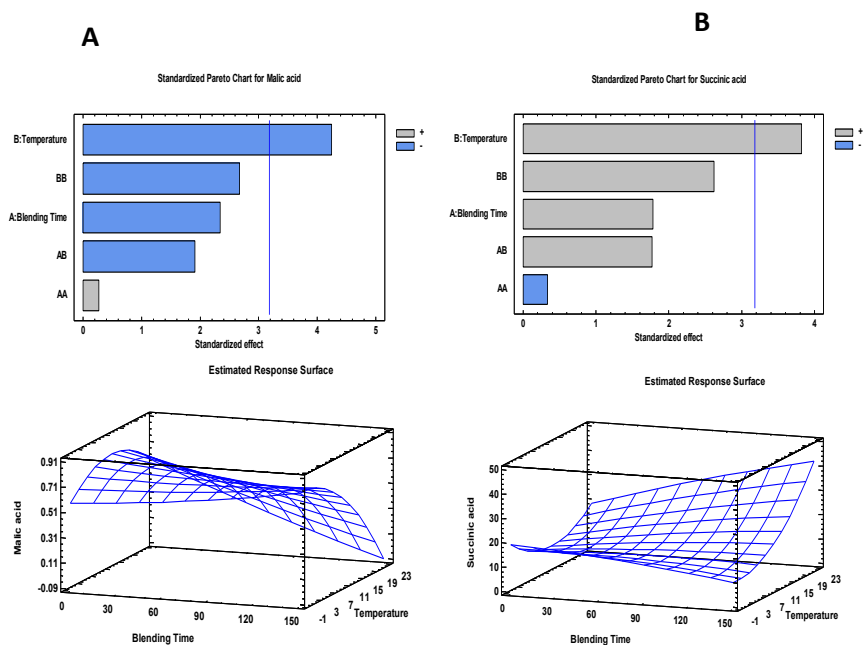


Figure 3.5 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on (A) malic acid (B) succinic acid (mg 100 g⁻¹ FW) of fresh-blended ‘Festival’ strawberry puree at Day 0.

Effects of blending time and temperature on dehydroascorbic acid and total anthocyanin content

Upon processing there were no significant differences in vitamin C content among the different blending time and temperature combinations applied. At Day 6, however, blending temperature had a significant effect on dehydroascorbic acid (DHA). As the blending temperature increased, the content of DHA significantly decreased (Figure 3.6). The vitamin C (AA+DHA) tends generally to reduce during storage. Since no significant differences in AA content were observed for puree samples, the reduction in DHA observed after 6 days of storage may also be related to the storage time. In fruits and vegetables under stress conditions, ascorbate oxidase has been described to promote the transformation of ascorbic acid (AA) into DHA (Wright and Kader, 1997; Tudela et al., 2002). During storage, temperature management is the most important factor to be taken into account in order to retain the initial AA content. AA is the predominant form of vitamin C present in fruits, and the primary oxidation product (DHA) is also important because it also has biological activity. Since the oxidized form is more prone to decomposition, leading to the loss of biological activity, the changes in AA forms are important in both technological and nutritional terms (Cordenunsi et al., 2005).

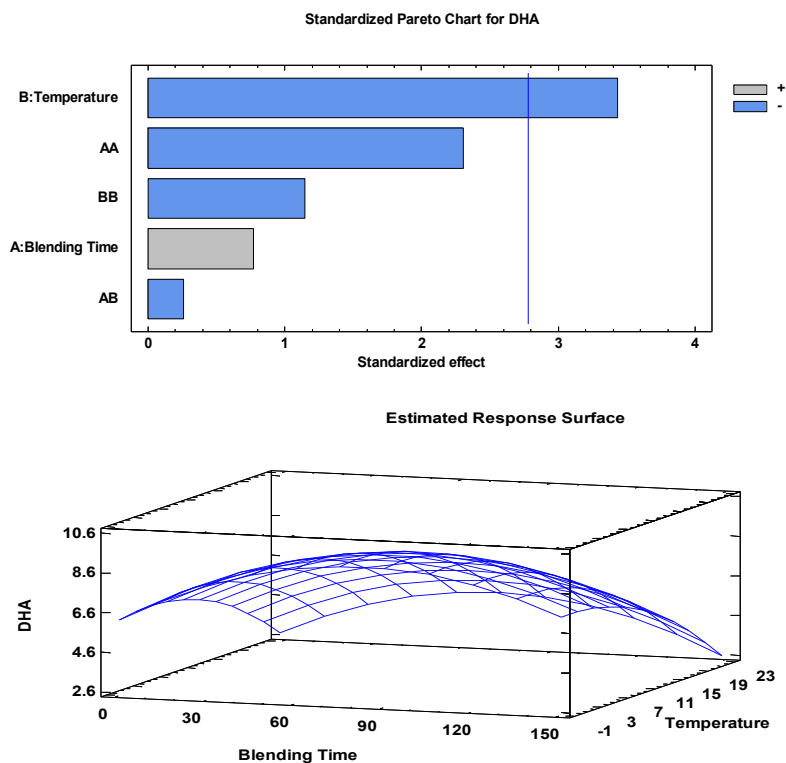


Figure 3.6 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on dehydroascorbic acid (DHA) content of fresh-blended ‘Festival’ strawberry puree at Day 6.

Blending temperature had a significant positive effect on the TAC. It seems that higher blending temperature (21°C) favors a higher TAC value (Figure 3.7). Maximum TAC values were observed when strawberries were blended at the highest temperature. At Day 0 5 seconds at 21 °C allowed to obtain 29.1 mg PG-3-glu 100 g-1 FW while 31.2 mg PG-3-glu 100 g-1 FW was determined when strawberry fruits were blended for 140 s at 21°C (Day 6). TAC seems to benefit from the high blending temperature, this was possibly due to the improvement in anthocyanin extraction caused by the higher temperature. Slight synthesis of

these compounds may also occurred during cold storage. A little increase at day 6 was in fact observed. Low temperatures could affect anthocyanin synthesis during storage of small fruits, inclusive of strawberries. It has been reported that anthocyanin biosynthesis and accumulation continue after harvest and during storage (Kalt et al., 1999). TAC is responsible for the red color of strawberry indicating that the higher the TAC the more intense was the puree color. This will make any change of color less apparent (Bursac Kovacevic et al., 2015). In the present work no significant differences in color parameters at day 6 were observed.

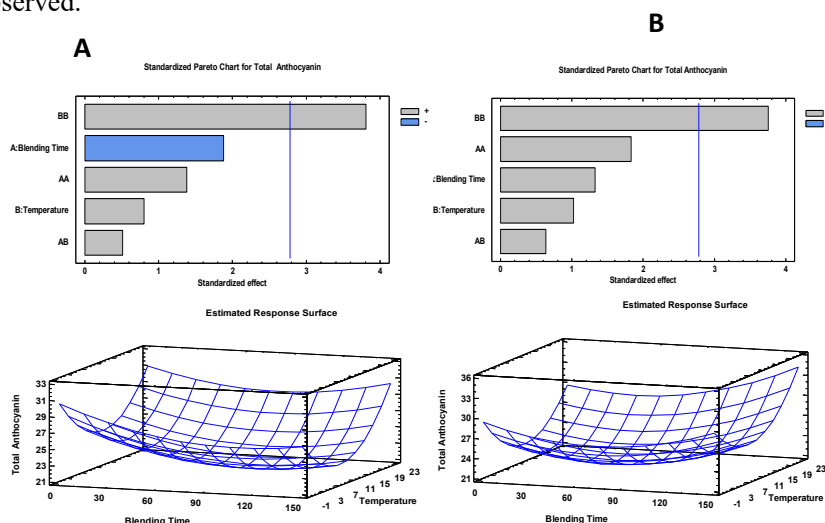


Figure 3.7 Standardized Pareto charts (top) and estimated surface responses (bottom) of blending time (s) and temperature (°C) factors on total anthocyanin content of fresh-blend strawberry puree (A) at Day 0 and (B) at Day 6.

TRIAL II

Results of two-way analysis of variance (Table 3.5) showed that blending temperature, storage time and their interaction significantly affected color parameters, vitamin C content (AA, DHA, and AA+DHA), total anthocyanin content, and sensory attributes (aroma and overall acceptance). Below these effects are described in detail.

Table 3.5 Effect of blending temperature (treatment) (5 or 21°C), storage time and their interactions on quality attributes of fresh-blended ‘Festival’ strawberry purees on some physicochemical and sensorial attributes of fresh-blended strawberry puree. Mean values of 12 samples (3 replicates x 4 sampling times); mean values followed by different letter (s) are significantly different ($P < 0.05$ according to Tukey’s test; (****) $P \leq 0.0001$; (***) $P \leq 0.001$; (**) $P \leq 0.01$; (*) $P \leq 0.05$; ns = not significant).

Quality attributes	5°C	21°C	Treatment (trt)	Storage time (t)	trt * t
<i>Physicochemical</i>					
L*	33.1 a	31.6 b	****	****	****
a*	26.8 ns	26.6 ns	ns	*	ns
b*	12.9 a	10.8 b	****	****	****
Chroma	29.8 a	28.7 b	***	****	****
Hue angle (h°)	68.0 a	64.0 b	****	****	****
Total color difference (ΔE^*)	6.3 b	7.7 a	*	**	**
Ascorbic acid; AA (mg 100 g ⁻¹ FW)	13.4 a	10.8 b	****	****	**
Dehydroascorbic acid; DHA (mg 100 g ⁻¹ FW)	43.2 a	41.7 b	*	****	ns
Total Vitamin C; DHA + AA (mg 100 g ⁻¹ FWf)	56.6 a	52.5 b	*	****	ns
Total anthocyanin content (mg PG-3-Glu 100 g ⁻¹ FW)	20.4 b	22.0 a	*	****	***
<i>Sensory attributes</i>					
Aroma	3.5 a	3.0 b	*	****	ns
Color	3.7 ns	3.6 ns	ns	****	ns
Homogeneity	4.1 ns	4.0 ns	ns	****	ns
Freshness	3.6 ns	3.3 ns	ns	****	ns
Sweetness	3.0 ns	2.9 ns	ns	****	ns
Acidity	3.2 ns	2.8 ns	ns	****	ns
Off-odor	1.8 ns	2.0 ns	ns	****	ns
Off-flavor	1.4 ns	1.6 ns	ns	****	ns
Overall acceptance	3.6 a	3.1 a	*	****	ns

Effect of blending temperature on color and total anthocyanin content

Mean values referred to all sampling dates shows that all color attributes (but a^* value) were significantly higher for samples blended at 5 °C (Table 3.5). Initially the average L^* , a^* and b^* values were 32.7, 33.1 and 28.7 and 33.1, 29.1, and 14.8 for blending at 5° and 21°C, respectively. However, trends over time for color attributes confirmed difference evident throughout storage but only significant at the second sampling day (8 days at 5 °C): puree obtained using blending temperature of 5°C resulted in significantly lower L^* , h° and ΔE^* values compared to the other treatment. No significant difference in color parameters was determined at the end of storage (see Figure 3.8 for L^* value and ΔE^*).

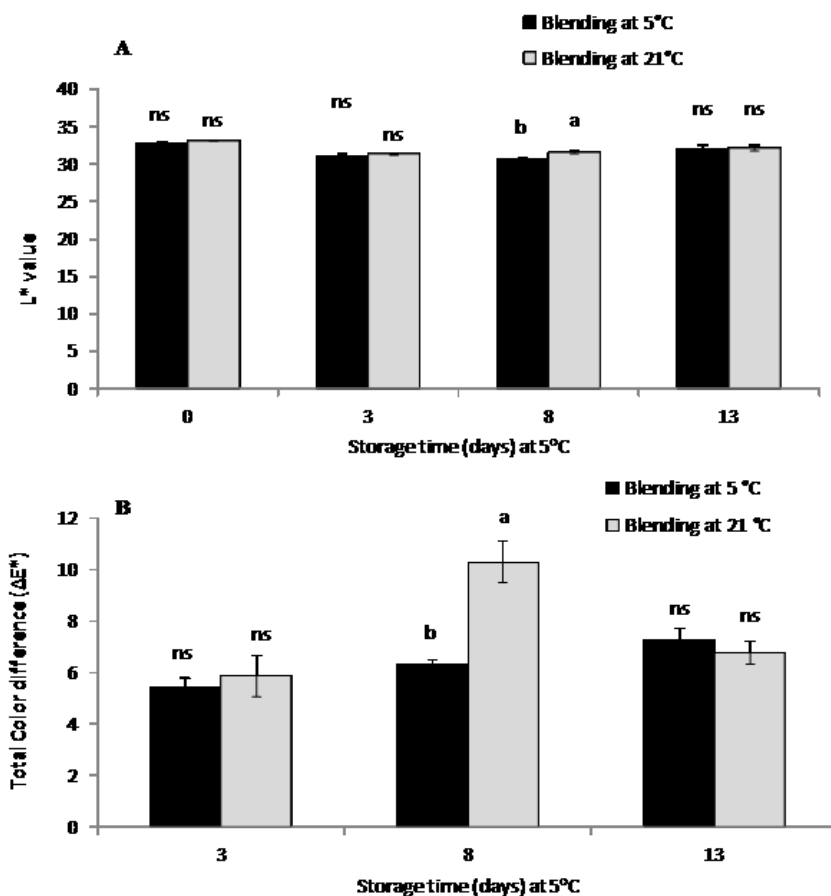


Figure 3.8 Effect of blending temperature on color parameters (A) L* value and (B) total color differences (ΔE^*) of fresh-blended strawberry purees storage for 13 days at 5 °C. mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey' s test (P value ≤ 0.05) and ns = no significance.

Mean values for total anthocyanin content (TAC) resulted higher in samples blended at higher temperature (Table 3.5) although they generally did not show a clear trend over time. In fact, upon processing TAC resulted significantly higher for samples blended at 5 °C, then they increased during the first 2 days of storage

(especially for samples blended at 21°C, which resulted significantly higher). TAC then decreased after 8 days (significantly higher for purees blended at 5 °C) and increased again at the end of storage, with content in samples blended at 21°C significantly higher than at 5 °C (Figure 3.9). The higher anthocyanin content indicates a greater color intensity of the product, that will make any change of color less apparent (Bursac Kovacevic et al., 2015). Similarly, Cao et al. (2012) found that ΔE^* values showed a negative correlation with anthocyanin and ascorbic acid contents in cloudy and clear strawberry juices. Therefore, the stability of both anthocyanins and ascorbic acid may influence the total color difference and results of the present study confirmed this behavior.

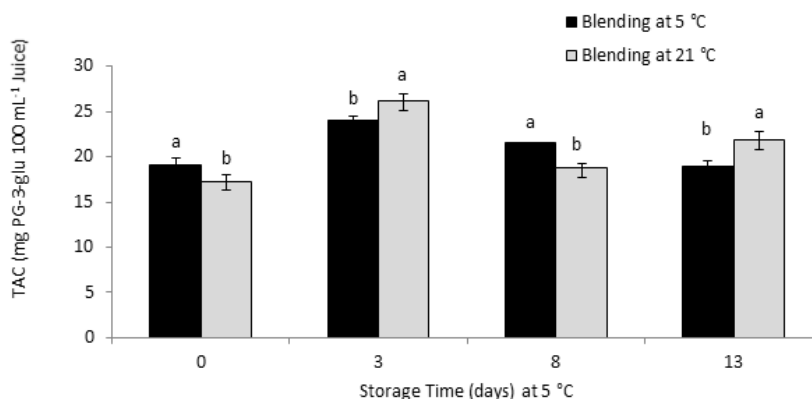


Figure 3.9 Effect of blending temperature on total anthocyanin content (TAC) of fresh-blended strawberry purees storage for 13 days at 5 °C. mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey' s test (P value \leq 0.05) and ns = no significance.

Effect of blending temperature on vitamin C content of fresh-blended strawberry puree

Samples blended at 5 °C presented higher mean values of contents in AA, DHA, and total vitamin C, compared to those blended at 21 °C. Over the storage time vitamin C showed a decreasing behavior (Figure 3.10). Initially there was no significant difference between the two blending temperatures. After 2 days, a significant reduction of ascorbic acid (AA) was observed, especially with sample blended at 21°C. Blending at 5°C was significantly better to retain the AA during storage up to the end of storage (Figure 10 A). On the other hand, dehydroascorbic acid (DHA) increased during the first 2 days of storage due to oxidation AA in to DHA. After 8 days at 5°C, blending at 21°C resulted in higher DHA content compared to blending at 5°C, clearly due to an enhanced oxidation of ascorbic acid, although no significant difference was observed at the end of storage (Figure 10 B). Vitamin C (as the sum of AA+DHA) maintained always higher in low-temperature blended samples, but at the second sampling date (8 days) when it results slightly higher in samples blended at 21 °C, although difference was not statistically significant (Figure 10 C). Loss of vitamin C is generally more rapid at higher temperatures (Watada and Weichmann, 1987). Like anthocyanins, the amount of AA is also dependent on the strawberry cultivar and ripening degree (Cordenunsi et al., 2002). To retain the initial AA content during storage, temperature management is the most important factor to be taken into account. Storage temperature resulted to be crucial to maintain a high AA

content (Lee & Kader, 2000). Results of the present work suggest that blending temperature is also of great importance in controlling AA concentration in fresh strawberry purees.

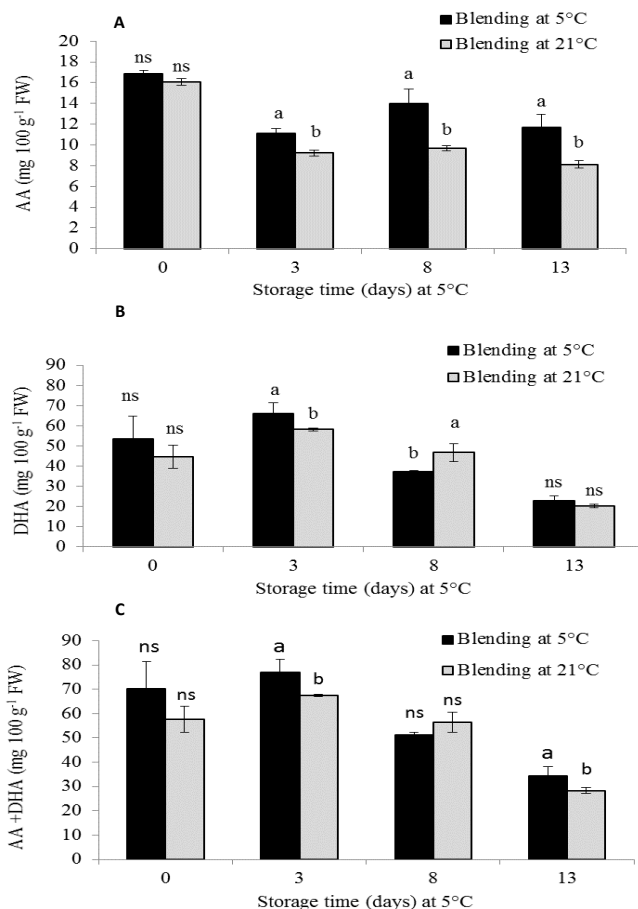


Figure 3.10 Effect of blending temperature on ascorbic (A) and dehydroascorbic acid (B) and total vitamin C (C) content of fresh-blended strawberry purees stored for 13 days at 5 °C: mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey' s test (P value ≤ 0.05) and ns = no significance.

Effect of blending temperature on sensory attributes of fresh-blended strawberry puree

During storage sensory attributes scores significantly decreased. Among the attributes evaluated by panelists scores for aroma (Figure 3.11 A) and for overall rating (Figure 3.11 B) showed significant difference between treatments. Immediately after processing, blending temperature did not significantly influence aroma nor overall rating scores. After 2 days of storage however, samples blended at 5 °C had significantly higher aroma and overall rating scores compared to those blended at 21 °C. Although no significant difference was determined after 8 days at 5 °C, samples were still acceptable showing aroma scores higher than 3 (limit of marketability) and overall rating scores slightly below this limit but still considerably above the limit of edibility (Figure 3.11 A and B). After 13 days, only samples blended at 5 °C were scored positively by panelists with scores higher than 2 for aroma. However, their overall rating was not evaluated, indicating a not edible product. Also for sensorial parameters, as commonly known, temperature management during processing and storage, results to be crucial. In sensory quality control not only the processing and storage temperatures but also the temperature of the product during analysis is equally important to influences result (Costell et al., 2010).

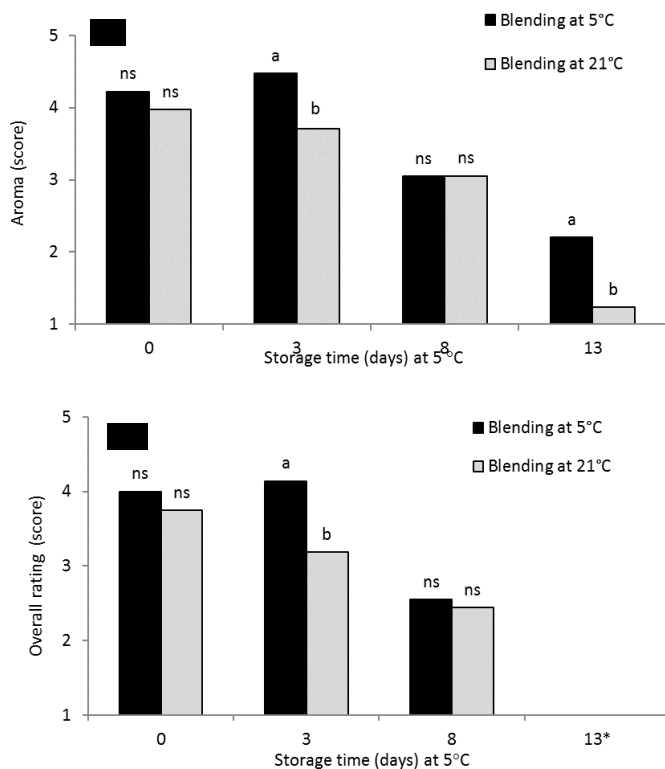


Figure 3.11 Effect of blending temperature on (A) aroma, (B) overall rating of fresh strawberry purees stored for 13 days at 5 °C: mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey's test (P value ≤ 0.05) and ns = no significance and * = not evaluated.

Conclusions

The effect of blending condition on quality attributes of freshly-blended strawberry purees was studied in this work. According to results, optimal blending conditions to obtain convenient puree from nutritional, organoleptic and technological points of view, include blending time lower than 90 s and temperature not exceeding 10°C. From a second trial results confirmed that blending at 5°C resulted in better color retention and higher vitamin C content, aroma and overall acceptance scores compared to blending at 21°C

Therefore, blending for approximately 90 s at low temperature (5 °C) could be a possible combination (within the range of studied conditions) for blending fresh strawberry purees with high quality. However, optimum blending conditions should be accompanied with appropriate storage and packaging conditions including packaging material in order to preserve quality and extend shelf life of fresh-blended strawberry-based products.

2.4 Effect of packaging condition on quality and shelf life of fresh-blended strawberry purees

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Abstract

The main objective of the study was to evaluate and compare the effect of packaging conditions of two plastic films under active and passive modified atmosphere packaging (MAP) to preserve quality of fresh-blended strawberry purees. 'Festival' strawberries were harvested from protected organic farming system in Mediterranean Agronomic Institute of Bari and stored at 5°C overnight in the postharvest laboratory (University of Foggia). Subsequently, uniform and defect free strawberries were homogenized in a blender at 4000 rpm for 90 s at 5°C. Two packaging materials, namely polypropylene/ethylene vinyl alcohol (PP/EVOH) and polypropylene/polyamine (PP/PA), were tested to package fresh-blended strawberry purees. Samples were packaged in 250 mL PP/EVOH plastic trays under active (5% O₂+13% CO₂) and passive (air) conditions. Physicochemical, sensorial, nutritional attributes and spoilage microbial counts were evaluated after 0, 2, 6, and 12 days of storage at 5°C. A two-way analysis of variance for MAP treatment, storage time and their interactions was run and significant means were separated by Tukey's test ($P < 0.05$). Results showed that after 12 days at 5°C, packaging significantly affected the physical and nutritional

attributes and microbiological counts. Samples in active MAP maintained slightly higher nutritional and sensorial attributes during storage. After 12 days at 5°C, samples in active PP/EVOH had lower microbiological counts ($< 4 \text{ Log CFU g}^{-1}$) and no perceived off-flavor compared to the other treatments. In conclusion, fresh-blended strawberry purees can be stored for about 12 days at 5°C using active PP/EVOH MAP condition for quality maintenance and freshness. Therefore, MAP combined with innovative non-thermal processing techniques may help in extending shelf life of fresh strawberry purees.

Key Words: ‘Festival’, fresh purees, Modified atmosphere packaging, PP/EVOH, PP/PA, shelf life

Introduction

The demand for fresh, hygienic and healthy food products has greatly increased with changes in consumer lifestyle and awareness and enhanced competition among fresh produce retailers. Fruits, vegetables and derived fresh products attract consumer attention as possess high levels of nutritional values (Rico et al., 2007). Strawberries are among the highly valued commodities in the fresh-cut industry for their attractive appearance and flavor, and possession of rich bioactive compounds such as vitamin C and anthocyanins.

The quality of fresh and minimally processed strawberries as a function of their physicochemical compositions and organoleptic attributes is an important area of study (Giuggioli et al., 2015). A major challenge in the production and marketing of fresh and minimally processed products is rapid quality deterioration and reduced shelf-life (Hussein et al., 2015). Modified atmosphere packaging (MAP) combined with refrigerated storage is widely and successfully used for safety maintenance and shelf-life extension of whole and minimally processed strawberries and has been the center of many studies (Castaigne et al., 1991; Shamaila et al., 1992; Renault et al., 1994; Van der Steen et al., 2002; Caner et al., 2008; Nielsen and Leufven, 2008). MAP is an active or passive dynamic process of altering gaseous composition within a package (Caleb et al., 2013). It relies on the interaction between the respiration rate of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Caleb et al., 2012b). MAP should contribute to reduce the respiration rate and enhance shelf life of produce providing optimum

combination of gases, generally low levels of O₂ and high levels of CO₂ (Sandhya, 2010). The choice of gas is very dependent upon the food product being packaged. In active MAP gases are deliberately displaced or replaced in the packages; scavengers and/or absorbers can be used to attain the desired concentrations. Whereas, in passive MAP all is demanded to film barrier properties with the objective to create the desired atmosphere (Lee et al., 1996). In a given package, important aspects to be considered are gas transfer rate (GTR), water vapor transfer rate (WTR), mechanical properties and transparency of the film, in addition to the type of package and sealing reliability. Obviously, produce physiology (such as the extrinsic and intrinsic factors affecting produce respiration rate) is extremely important in packaging development phases (Caleb et al., 2013). Plastic films used in MAP for respiring fresh produce are permeable to gases and the degree of permeability depends on the chemical composition and thickness of film as well as on temperature. Other films are instead designed to prevent the exchange of gases and are mainly used with non-respiring products (Thompson, 2010a). However, some problems related to the inappropriate use of MAP conditions for fresh produce can occur leading to development of anaerobic conditions and/or too high CO₂ levels which eventually have a detrimental effect on the product quality through production of ethanol, acetaldehyde and off-flavor and odors (Saltveit, 2003).

For freshly blended strawberry purees, considering their very low respiration rate (see previous experiment), it can be interesting to evaluate the application of high

barrier packaging materials. PP/EVOH and PP/PA can be useful for their excellent gas barrier properties and resistance to organic solvents and water resistance properties. The recommended gas mixtures for extending shelf life of strawberries range between 5-10% O₂ and 15-20% CO₂ in nitrogen (Sandhya, 2010). When strawberries are blended into purees, the respiration rate significantly reduced and this may represent an advantage to be exploited to design appropriate MAP conditions.

Therefore, the main objective of the present work was to study and compare the effect of different packaging conditions on quality and shelf life of fresh-blended strawberry purees. PP/EVOH and PP/PA plastic films under both active (5% O₂ and 13% CO₂) and passive (air) modified atmosphere packaging were evaluated. The main focus was to select a packaging condition that can better maintain the physicochemical, sensorial and nutritional quality attributes and retards spoilage microbial growth during storage of 12 days at 5°C.

Materials and Methods

Sample preparation

‘Festival’ strawberries (*Fragaria x ananassa* Duch.) grown under protected organic farming system at the Mediterranean Agronomic Institute of Bari (MAIB) experimental field, Apulia region (South Italy) were harvested at commercial maturity, transported into the postharvest laboratory (University of Foggia) and stored at 5°C overnight. Uniform color and shape, free of physical defects and decay strawberries were visually selected and categorized into three

groups; washed with tap water and dried on a paper towel. After calyx removal, fruits were divided into two halves. Subsequently, about 1 kg of fruit slices (for each replicate) were blended at high power food Blender (Bimby TM31, Vorwerk, Germany) using 4000 rpm for 90 s at 5°C and samples (200 mL) were packaged in 250 mL polypropylene/ethylene vinyl alcohol/polypropylene (PP/EVOH/PP) rigid plastic trays sealed by PP/EVOH (70 µm) or PP/PA (60 µm) flexible plastic films under active (5 % O₂ + 13% CO₂) and passive (air) MAP conditions. The active MAP condition was chosen on the basis of the optimal gaseous mixture to be applied during storage of strawberry-based products (Sandhya, 2010). Physicochemical, sensory, nutritional attributes and spoilage microbial counts were evaluated after 0, 2, 6, and 12 days of storage at 5°C. All samples were prepared in triplicates. Samples were immediately analyzed after each sampling times and when it was necessary, samples were frozen at -80°C until analysis.

Gas composition

The oxygen and carbon dioxide percentage inside the packages was measured in 15 mL headspace gas sample using a handheld gas analyzer (CheckMate 3 O₂/CO₂, Dansensor A/S, Denmark) during each sampling time.

Color and viscosity of puree

A spectrophotometer (CM-2600d Konica Minolta, Japan) was used to measure color of the puree sampled on a Petri dish during each sampling times, obtaining L*, a*, and b* values. Moreover, Chroma (C*) and hue angle (h°) parameters

were calculated using equations 1 and 2, respectively. In addition, total color differences (ΔE^*) of purees was calculated at each sampling time in reference to the initial values (equation 3) as described by Ayala-Zavala et al. (2004):

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$h^\circ = \arctan (b^*/a^*) \quad (2)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

According to Ayala-Zavala et al. (2004) the total color difference or ΔE^* value is the change between the initial and final measure and it is estimated as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0).

Rheological measurements were performed with a rotational Brookfield LV, DV-II-Pro viscometer (Harlow, England). Approximately, 50 g of each sample were placed in the concentric cylindrical cup. For the analysis the spindle No. 3 was used, applying a speed equal to 40 rpm for 30 s at a room temperature. The viscosity was expressed in centipoise (cP).

Sensory evaluation

Sensory analysis was carried out by a 6-member expert panelist consisting of University staff who were trained and familiar with the characteristics of the product. Panelists were asked to rate samples for aroma, color, homogeneity, freshness, sweetness, acidity, off-odor, off-flavor and overall acceptance, on a 5-point hedonic scale from values ranging from 1 as minimum to 5 as a maximum. Samples were coded with random three-digit numbers in identical plastic cups

and order of presentation to each panelist was randomized. Each sample was replicated 12 times (2 replicates per treatment and 6 panelists). A score of 3 was considered as a limit of commercial acceptability and 2 as the limit of consumption.

Total soluble solids, titratable acidity, pH-value, and sugar/acid ratio

Total soluble solids (TSS) was obtained by measuring the refractive index of strawberry juice using a digital refractometer (Atago RX-7000cx; Atago Co. Ltd., Japan) at 25°C. Some drops of juice were placed on the lens and reading was noted in degree Brix (°brix). Calibration was made with distilled water and the lens was carefully rinsed between samples. One gram of sample was used to measure the pH and the titratable acidity (TA), with an automatic titrator (T50 M Terminal, Mettler Toledo, Switzerland). TA was obtained by measuring the volume of 0.1N NaOH used to reach a final pH of 8.2. All determinations were made in triplicate and the results were expressed as grams of citric acid equivalent per 100 g fresh weight as described by Mahmood et al. (2012). Moreover, the pH value of the puree was measured by a calibrated pH-meter (pH 4, pH 7). The sugar/acid (TSS/TA) ratio was calculated by dividing the soluble solid contents values by the titratable acidity.

Vitamin C, Total phenolic and anthocyanin contents, and antioxidant capacity

To measure vitamin C content, 5 grams of strawberry purees mixed with 10 mL of methanol-water (5:95, v/v), plus citric acid (21 g L⁻¹), EDTA (0.5 g L⁻¹) NaF (0.168 g L⁻¹) was homogenized using an Ultraturrax for 1 min. The homogenate

was filtered through cheesecloth and the pH was adjusted to 2.2–2.4 by addition of 6 mol L⁻¹ HCl. After that the homogenate was centrifuged at 10000 rpm for 5 min. Supernatant was taken and filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then through a 0.2 µm cellulose acetate filter. L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA) contents were determined as reported in Zapata and Dufour (1992). The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1, 2-dihydroxyethyl) furol [3, 4-b] quinoxaline-1-one (DFQ) with 1, 2-phenylenediamine dihydrochloride (OPDA). Samples of 20 µL were analyzed with an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 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 mmol L⁻¹ cetrimide and 50 mmol L⁻¹ potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min⁻¹. The detector wavelengths were 348 nm for DHA and 251 nm for AA. AA and DHA contents were expressed as mg of L- ascorbic or L-dehydroascorbic acid per 100 g of fresh weight (mg 100 g⁻¹ FW).

Total phenolic content (TPC) was analyzed by using the Folin–Ciocalteu method (Singleton and Rossi, 1965b). Five grams of purees were homogenized in an Ultraturrax after adding 10 mL of extraction buffer containing 200 mL of distilled water, 800 mL of methanol and 2 mM (84 mg L⁻¹) of sodium fluoride

(NaF) as described by Singleton and Rossi (1965). Extracts were then centrifuged at 5°C and 9000 rpm for 10 min. Extract of 100 µL was mixed with 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L⁻¹). Then samples were left at room temperature for 2 h in the dark. The absorbance was read at 725 nm against a blank (prepared in same way except the 100 µL of samples was replaced by 100 µL distilled water) using a spectrometer (UV- 1700 Shimadzu, Jiangsu, China). TPC was calculated based on the calibration curve of gallic acid and results were expressed as milligram of gallic acid equivalents per 100 g fresh weight (mg GAE 100 g⁻¹ FW).

To measure TAC, one gram of strawberry sample was mixed with 20 mL of extraction solution (methanol in 1% hydrochloric acid; HCl-MeOH mixture) and homogenized for 20 s using an Ultraturrax. The homogenate was then centrifuged for at 4°C at 2000 rpm, 15 min. Then 700 µL of supernatant plus 300 µL of 1% HCl-MeOH solution were put in cuvettes and total anthocyanin content was read immediately in spectrophotometer at 510 nm as described by Cordenunsi *et al.* (2002). Results were expressed in mg of pelargonidin-3-glucoside equivalents per 100 g of fresh weight (mg PG-3 glu 100 g⁻¹ FW).

The antioxidant capacity (AC) assay was performed on same extracts used for total phenolic contents following the procedure described by Brand-Williams *et al.* (1995), with little modifications where diluted sample of 50 µL was pipetted into 950 µL of DPPH (2, 2-Diphenylpicrylhydrazyl) solution and absorbance was

read after 24 h at 515 nm. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) was used as a standard and results were expressed in milligrams of Trolox equivalents per 100 g of fresh weight (mg TE 100 g⁻¹ FW).

Microbiological analysis

Microbiological analysis was conducted according to the methods reported by Nogales-Delgado *et al.* (2013) with slight modifications. Triplicate samples were subjected to microbial analysis for each treatment during each sampling time. Ten grams of sample were homogenized in 90 mL sterile physiological saline solution (0.85% NaCl) homogenized in a stomacher (BagMixer 78860, ST-Nom Interscience, Saint Nom, France) for 60 s. Colony forming unit (CFU) of aerobic mesophilic (30°C for 2 days) and psychrophilic (5°C for 10 days); yeast and molds (25 °C for 5 days) were counted in Plate Count Agar (PCA), Potato Dextrose Agar (PDA), respectively; microbial populations were expressed as Log CFU g⁻¹.

Statistical analysis

Two-way ANOVA with treatment and storage time as factors and their interactions were run to analyze the data. Significant means were separated by the Tukey's test; $P < 0.05$) using the Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA USA) software. Moreover, the effect on quality parameters of treatment was tested by performing a one-way ANOVA for each sampling day, and mean values within each sampling were separated applying Tukey test with significant difference when $P \leq 0.05$.

Results and Discussion

Two-way analysis of variance on the effects of packaging conditions (treatments), storage time and their interactions revealed significant differences on some physicochemical, nutritional, sensorial attributes and spoilage microbiological counts. Packaging condition had significant effects on L*, a*, Chroma, h°, ΔE^* , viscosity, TSS, AA, DHA, total vitamin C, TPC, TAC, AC, off-flavor and microbiological count. Storage time significantly affected physical attributes of the purees, TSS, AA, DHA, vitamin C, TPC, TAC, sensorial attributes, mesophilic bacteria and yeast & molds. Whereas, only the viscosity, TSS, AA, TPC, TAC, off flavor and microbiological contamination were significantly affected by their interaction (Table 4.1).

Table 4.1 Effect of packaging condition (PP+EVOH and PP+PA plastic films under active and passive MAP), storage time and their interactions on quality attributes of fresh-blended ‘Festival’ strawberry purees. Mean values of 9 samples (3 replicates x 3 sampling times); mean values followed by different letter (s) are significantly different ($P < 0.05$ according to Tukey’s test; (****) $P \leq 0.0001$; (***) $P \leq 0.001$; (**) $P \leq 0.01$; (*) $P \leq 0.05$; ns = not significant).

10	PP/EVOH active	PP/EVOH passive	PP/PA active	PP/PA passive	Treatment (trt)	Storage time (t)	trt x t
Physical attributes							
L*	29.5 ab	29.4 b	29.9 a	29.2 b	**	****	ns
a*	20.3 ab	20.5 ab	21.0 a	19.7 b	*	****	ns
b*	9.3 ns	9.4 ns	9.3 ns	9.2 ns	ns	****	ns
Chroma	22.3 ab	22.6 ab	23.0 a	21.8 b	*	****	ns
Hue angle (h°)	65.4 ab	65.4 b	66.1 a	64.9 b	***	****	ns
Total color difference (ΔE^*)	7.9 b	8.2 b	7.9 b	9.0 a	**	****	ns
Viscosity (Centipoise (cP))	1036.7 b	1105.8 a	998.8 c	1080.8 a	****	****	*** *
Chemical attributes							
Total soluble Solids (TSS) (°Brix)	7.9 c	8.0 b	8.3 a	7.8 c	***	ns	*
pH value	3.7 ns	3.6 ns	3.7 ns	3.6 ns	ns	****	ns
Titrateable acidity (TA) (g citric acid 100 mL ⁻¹ juice)	0.7 ns	0.7 ns	0.7 ns	0.7 ns	ns	ns	ns
TSS/TA ratio	11.0 ns	11.1 ns	11.2 ns	10.9 ns	ns	ns	ns
Ascorbic acid (mg 100 g ⁻¹ FW)	7.9 ab	5.4 c	9.0 a	7.2 b	****	****	***
Dehydroascorbic acid (mg 100 g ⁻¹ FW)	85.1 a	87.3 a	70.5 c	78.1 b	****	****	ns
Total Vitamin C (mg 100 g ⁻¹ FW)	93.0 a	92.7 ab	79.6 c	85.3 bc	***	****	ns
Total Phenolic Content (mg GAE 100 g ⁻¹ FW)	171.1 b	175.7 ab	183.6 ab	188.8 a	**	****	***

Total anthocyanin content (mg PG-3-glu 100 g ⁻¹ FW)	21.0 b	24.5 a	21.8 b	21.2 b	**	**	*
Antioxidant capacity (mg TE 100 g ⁻¹ FW)	164.4 b	194.9 a	176.2 ab	198.5 a	*	ns	ns
Sensorial attributes (Score)							
Aroma	3.7 ns	3.2 ns	3.6 ns	3.1 ns	ns	****	ns
Color intensity	3.7 ns	3.7 ns	3.8 ns	3.6 ns	ns	****	ns
Homogeneity	3.9 ns	4.0 ns	3.7 ns	3.8 ns	ns	****	ns
Freshness	3.7 ns	3.4 ns	3.5 ns	3.1 ns	ns	****	ns
Sweetness	3.1 ns	3.2 ns	3.2 ns	3.0 ns	ns	****	ns
Acidity	2.7 ns	2.9 ns	3.0 ns	2.9 ns	ns	****	ns
Off-odor	1.6 ns	1.7 ns	1.7 ns	2.2 ns	ns	****	ns
Off-Flavor	1.1 c	1.9 ab	1.6 b	2.0 a	****	****	*
Microbiological count (Log CFU g⁻¹)							
Aerobic mesophilic bacteria	3.5 b	3.8 ab	3.7 ab	3.9 a	**	****	***
Psychrophilic bacteria	2.2 c	4.0 a	3.4 b	4.1 a	****	ns	***
Yeast & molds	3.8 b	3.7 b	4.0 b	4.4 a	***	***	***

Effect of packaging conditions on gas composition

Gas concentrations within a sealed plastic package headspace change due to food metabolism, microbial respiration, and permeability of the packaging material (Simpson *et al.*, 2009). Initially the gas composition was 5% O₂ and 13% CO₂ in active MAP and air in the passive treatments in both PP/EVOH and PP/PA packaging materials. The effect of packaging condition on O₂ and CO₂ evolution of fresh-blended strawberry purees packaged in active and passive MAP in PP/EVOH or PP/PA during 12 days' storage at 5°C is shown in Figure 4.1. The initial CO₂ concentration in the active packages changed by approximately 50% just after 2 days of storage regardless of the packaging material and presumably reached the equilibrium levels. These trends were maintained throughout time and, at the end of storage 5.5 and 5.6% of CO₂ were measured in PP/EVOH and PP/PA active samples, respectively. On the other hand, level of O₂ remained constant around 5% throughout storage time, showing that O₂ consumption was roughly equated by O₂ entering the packages. In passive MAP this equilibrium was not reached up to the end of storage time. However, it was possible to notice that O₂ percentage tended to decrease and, on the other hand, CO₂ increased. In all probability, if time was allowed, a possible stationary phase of 5% O₂ + 6% CO₂ concentration may be expected to be reached. In general, the two packaging materials did not have a significant difference in terms of gas evolution.

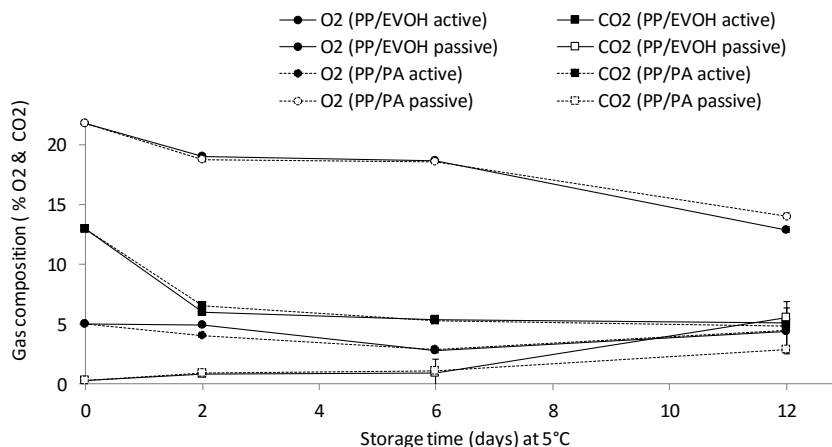


Figure 4.1 Effect of packaging condition (PP/EVOH and PP/PA active (5% O₂+13% CO₂) and passive (air) MAP on O₂ and CO₂ gas composition (%) of fresh-blended ‘Festival’ strawberry purees storage during 12 days at 5°C; mean values (n=3) and standard errors bars.

As already reported, the recommended gas mixtures for extending shelf life of strawberries is approximately 5-10% O₂ and 15-20% CO₂ in nitrogen (Sandhya, 2010). Anyhow, strawberry purees show a reduced respiration rate if compared to whole fruits. For this reason, the application of an active MAP (using as starting concentrations 5% O₂ and 13% CO₂) to store these products should represent an advantage. Results of this work suggest that active condition allowed to preserve the optimal O₂ concentration within the package up to the end of storage time. At the same time however, target of high CO₂ maintenance throughout the entire storage period was not reached.

Effect of packaging condition on color and viscosity

As already shown in Table 4.1, the packaging condition and storage time significantly affected the L^* , a^* , b^* , Chroma, hue angle (h°), and the ΔE^* values. Their interaction however did not have significant effects on any of the color parameters. L^* values of strawberry purees in PP/PA active packaging was the highest (Table 4.1), followed by active PP/EVOH packaged samples, and was significantly different from PP/PA passive and PP/EVOH passive packaging which exhibited lower L^* values, indicating that passive atmosphere packaging was darkening the colour of strawberry purees probably due to high oxygen concentration (Figure 4.1) which may have gradually favoured oxidative browning. The characteristic red colour of strawberry represented by the a^* value was highest in PP/PA active packaging and corresponding to L^* value, lowest in PP/PA passive packaging treatment, however, b^* values showed no significant difference among the treatments. Consequently, hue angle and Chroma were highest in PP/PA active and the least in PP/PA passive packaging treatment. Low values in hue angle and Chroma in this case reflects loss of red colour and change of colour to reddish-brown respectively during storage. Change in total colour variation values (ΔE^*) were higher in PP/PA passive atmosphere, with least scores in PP/PA active and PP/EVOH active atmosphere storage. High changes in colour, low hue angle, saturation index/Chroma, a^* value and L^* passive atmosphere storage could due be to higher O_2 concentration, as the elimination of this gas during processing has been found to improve colour characteristics of strawberry purees (Al-Maiman, 1997).

Evolution over storage time in L^* and ΔE^* values showed changes for all packaging conditions (data not shown). L^* values of the purees showed a general decrease during

the first 2 days and increase afterwards. After 2 days of storage, the PP/PA active conditions showed a less noticeable reduction resulting in significant higher L^* values compared to the rest of the treatments. In the PP/EVOH packages the average L^* value reduced from 30 to approximately 27, without significant difference between active and passive MAP. After 6 days at 5°C, the L^* value started to increase and the PP/PA active MAP continued to maintain a significantly higher L^* value. After 12 days of storage, PP/PA passive had significantly lower L^* value compared to the rest of treatments. The total color difference of strawberry purees (ΔE^*) increased during the first 6 days and was maintained afterwards, during storage for 12 days at 5°C. All samples showed a noticeable total color difference already after 2 days of storage, with maximum values in the PP/PA passive packages ($\Delta E^*=7$). However, differences among treatments were statistically significant only after 6 days of storage. At the same time PP/PA active condition allowed to better retain the initial product color, having after six days the lowest ΔE^* value. On the other hand, this difference was not to be seen anymore at the end of storage.

In general, looking at color parameters it was possible to notice that storage in active PP/PA was the best condition for color attributes preservation of fresh purees. The samples presented in fact highest L^* value, that meant the least browning occurrence in the product. A brighter and more saturated red color of active PP/PA samples was indicated by higher hue angle and Chroma values, respectively. Everything was confirmed by the significantly lower total color variation after 6 days of cold storage. The color changes of the purees were related to the gas evolution over time. As commonly known, elevated CO_2 concentrations or reduced O_2 in the active MAP

conditions limit color changes by delaying browning. According to Kader (2002b), CO₂-enriched atmospheres can have beneficial effects in delaying browning in some fresh-cut fruits and vegetables while reduced oxygen concentration allows to avoid tissues oxidation and the resulting damage. As for the present work, difference in O₂ percentage within the samples was even very high, as in passive MAP its level always were similar or slightly lower than the atmospheric composition, in the active MAP it was maintained at 5% and this limited oxidation preserving higher lightness and lower color variation.

Packaging conditions, storage time and their interaction showed significant effects on viscosity of the fresh-blended strawberry purees during the storage time. Generally, samples in passive MAP presented significantly higher values, regardless of the packaging material, while samples in active MAP with PP/PA shows the lowest average viscosity (998.8 cP) (Table 4.1). Figure 4.2 shows viscosity evolution of fresh strawberry purees over time. During the first 6 days of storage, packaging in passive PP/EVOH resulted in higher viscosity values compared to the rest of the treatments while at the end of the storage, the highest value was found with the PP/PA passive (that ranged from 827.5 of initial day to 1197.5 cP * 30 s⁻¹). All active MAP packages showed significantly lower values, indicative of lower changes from the initial values and therefore better quality maintenance (Figure 4.2). Viscosity is an important parameter to be considered in puree production, since it influences consumer acceptance. It is related to the presence of pectolytic enzymes like pectin methyl esterase (PME) and polygalacturonase (PG) in the raw material among other factors. Generally, viscosity decreases during storage mainly due to inactivation of PME and PG enzymes during processing (Aguilo-Aguayo *et al.*, 2009). However, oxygen percentage in the atmosphere surrounding the product

could negatively affect enzyme activity. Kanellis and Solomos (1985) observed that during the first days of storage air storage could inhibit PME activity leading to a higher viscosity determination if compared with low-O₂ application.

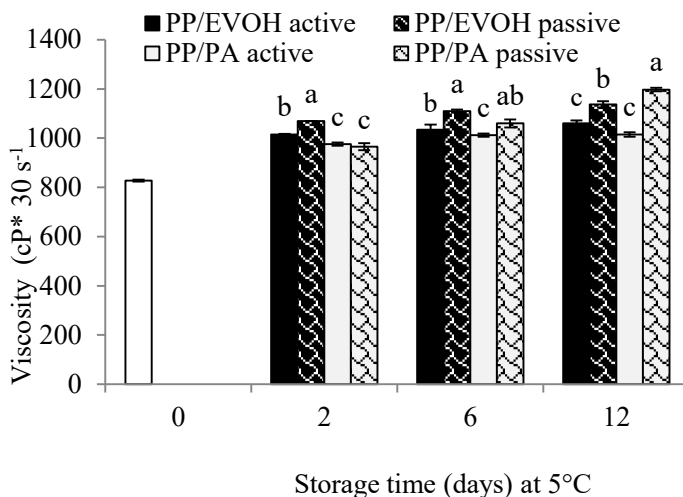


Figure 4.2 Effect of packaging conditions (PP/EVOH and PP/PA active (5% O₂+13% CO₂) and passive (air) on viscosity of fresh-blended ‘Festival’ strawberry purees stored for 12 days at 5°C; mean value and standard errors (n=3); Means with different letters at the same storage time are significantly different according to Tukey’ s test (P value \leq 0.05) and ns = no significance.

Sensory attributes

The sensory quality of strawberry is the result of a complex balance among sweetness, aroma, texture, and appearance (Jouquand et al., 2008). Storage time affected significantly all the sensorial attributes evaluated by panelists, while package conditions and its interaction with time showed significant effect only on off-flavor. This parameter was highly perceived for passive conditions. It was moreover observed that active MAP samples had slightly higher aroma (score approximately equal to 3) and overall acceptance (score higher than 2.6) and low off-flavor compared to the PP/PA passive samples which induced off-flavor development probably due to microbial growth with the passive MAP samples (Figure 4.3). It is important to notice that color differences as evaluated instrumentally, were not confirmed by panelists, as no differences were

observed in mean color score related to all sampling dates, nor there was any significant difference perceived among treatments at any given sampling time.

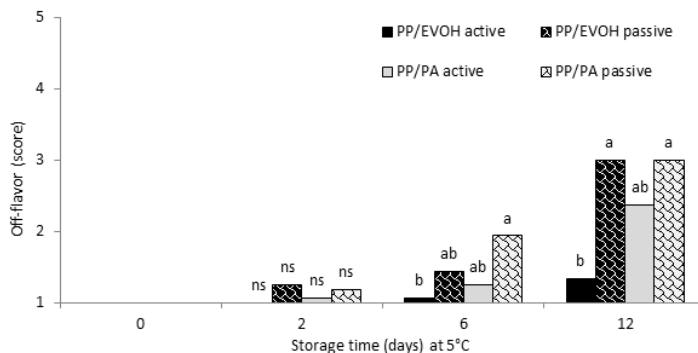


Figure 4.3 Effect of packaging conditions (PP/EVOH and PP/PA active (5% O₂+13% CO₂) and passive (air) on off-flavor score of fresh-blended ‘Festival’ strawberry purees stored for 12 days at 5°C; mean value and standard errors (n=21); Means with different letters at the same storage time are significantly different according to Tukey’s test (P value ≤ 0.05) and ns = no significance.

Total soluble solids, titratable acidity, pH-value and TSS/TA ratio

Total soluble solid (TSS) was significantly influenced by treatment ($P < 0.001$) and interaction of treatment and storage time ($P < 0.05$) but not by storage time. The effect of packaging conditions on TSS values of fresh-blended strawberry puree did not show a clear trend (see Table 4.1): samples in active PP/PA showed the highest mean values, significantly different from samples in active PP/EVOH and in passive PP/PA, but not from those in passive PP/EVOH. Trends of time confirmed this situation up to the end of storage (data non shown). Titratable acidity, pH, and TSS/TA ratio were not significantly affected by packaging conditions and by time of storage, nor by their interaction (see Table 4.1) although a slight increase in pH value was observed during storage at 5 °C. The average pH values ranged between 3.6 and 3.7 and TA was equal to

0.7 g citric acid 100 mL⁻¹ juice. TSS/TA ratio was approximately 10.9-11.0 in the passive and active MAP samples, respectively.

Vitamin C, total phenolic and anthocyanin contents and antioxidant capacity

Strawberries are of great interest because they are a good source of bioactive compounds including vitamin C, anthocyanins and flavonols. Among fruits, they have one of the highest antioxidant activities (Cordenunsi et al., 2005). In the present study packaging condition, storage time, and their interaction significantly affected the ascorbic acid (AA) of fresh-blended strawberry purees, while the single factors showed an effect on both DHA and total vitamin C (Table 4.1). Looking at mean values for all sampling dates, treatment in active PP/PA showed the highest value in AA and the lowest value in DHA. On the other hand, the lowest value in AA and highest in DHA was observed in samples stored in passive PP/EVOH MAP conditions. Among other treatments, it is noticeable that samples in active PP/EVOH MAP showed a good content of AA (somewhat lower, but not statistically significant, from active PP/PA, but the highest level of the oxidized form DHA, and the highest level of the combined AA+DHA forms, indicated in Table 4.1 as total vitamin C.

Trends over time for all packaging conditions are shown in Figure 4.4. After 2 days, almost 3-fold reduction of AA was observed; for instance, in passive PP/EVOH samples AA decreased up to 10.3 mg, starting from 26.3 mg 100 g⁻¹ FW (Figure 4.4 A). Throughout storage the total AA of active MAP strawberries was less reduced when compared with passive MAP fruit, this indicating a beneficial effect of low oxygen and high carbon dioxide atmosphere application. Substantial depletion of vitamin C content has been found in fresh-cut strawberries through the storage time irrespective of the

initial in-package atmosphere (Odrizola-Serrano et al., 2010). The magnitude of the depletion in vitamin C may be related to the O₂ concentration inside the packages (Soliva-Fortuny et al., 2002). In general, low O₂ atmospheres reduce deterioration and losses of AA in fresh produce. Elevated CO₂ atmospheres up to 10% also reduces AA losses, while higher CO₂ concentrations can accelerate these losses (Kader, 2002b). Ascorbic acid reduction is mainly due to its oxidation into DHA (Agar *et al.*, 1999) (Figure 4.4 B). The latter showed in fact an increasing behavior, almost similar for all samples. As a result, the total vitamin C (AA+DHA) increased after 2 days of storage (Figure 4.4 C) but it decreased after prolonged storage, mostly because of mechanical and thermal treatments. DHA represented the largest part of total vitamin C, as it was also found by other authors (Davey *et al.*, 2000; Lee and Kader, 2000). Relatively higher AA retention was found with the active MAP and the oxidized form increase was found less consistent in PP/PA treatments, especially in active PP/PA MAP conditions. However, the amount of DHA produced in all samples was much higher than the amount of AA oxidized. In order to explain this trend in strawberry cultivars, some authors observed that DHA evolution during storage, when associated with ascorbic acid retention, can be taken as an evidence of an operative redox system (AA/DHA) acting during cold storage, and there seems to be no fruit-specific pattern of total AA or DHA accumulation, but a cultivar-specific one (Cordenunsi *et al.*, 2005).

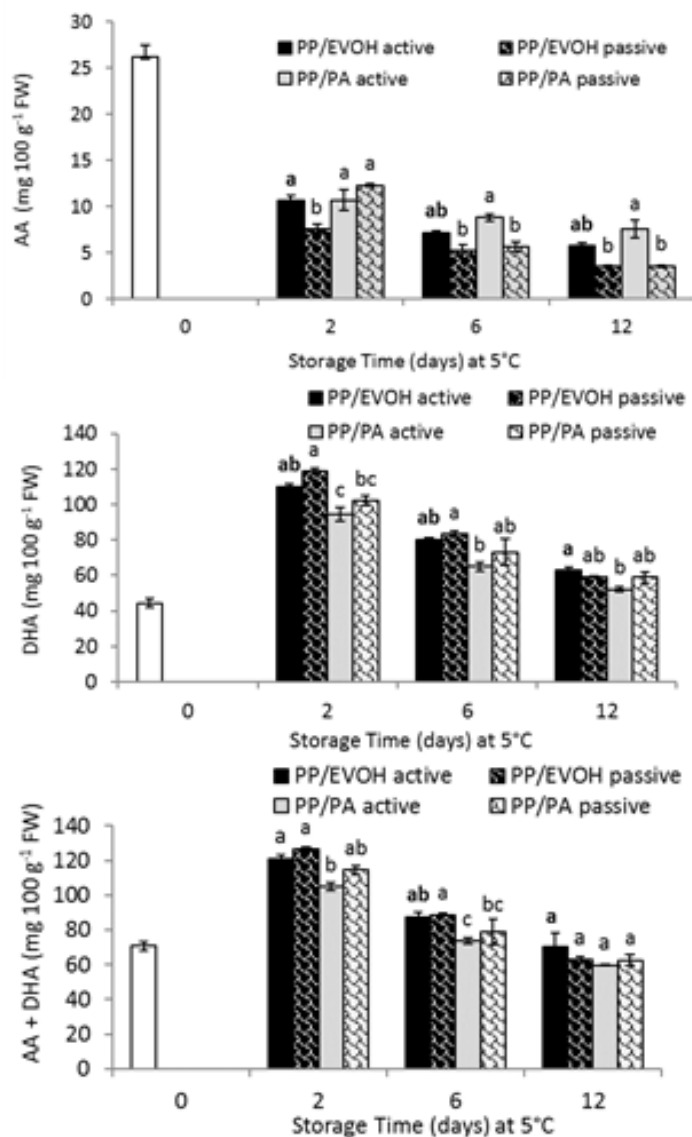


Figure 4.4 Effect of packaging condition (PP/EVOH and PP/PA active (5% O₂+13% CO₂) and passive (air) on (A) ascorbic acid (AA), (B) dehydroascorbic acid (DHA), and (C) total vitamin C (AA +DHA) of fresh-blended ‘Festival’ strawberry purees stored for 12 days at 5°C; mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey’s test (P value ≤ 0.05) and ns = no significance.

Packaging conditions, storage time and their interactions significantly affected ($P < 0.05$) both total phenolic (TPC) and total anthocyanin contents (TAC) of the purees, whereas only packaging conditions had significant effect on antioxidant capacity (AC). With reference to all sampling times, lowest mean value of TPC is observed in samples stored in active PP/EVOH MAP conditions, while all other treatments did not show significant differences among each other, with fairly higher values in samples stored in PP/PA (see Table 4.1).

The initial TPC of the purees was found to be 196.9 mg GAE 100 g⁻¹ FW and after 2 days, PP/EVOH passive had significantly higher TPC compared to other treatments while PP/PEVOH active and PP/PA passive conditions showed the lowest TPC values. After 6 days, samples packaged in the PP/PA films showed significantly higher TPC than the PP/EVOH films. The PP/EVOH active MAP showed relative lower values throughout the whole storage period. After 12 days of storage, however, the PP/PA passive presented the highest TPC although considerable high TPC was maintained in all the treatments (Figure 4.5 A). In fact, combination of gas used is also reported to prevent phenol losses in stored shredded carrots (Amanatidou et al., 2000) and strawberries (Bhat and Stamminger, 2015). In strawberries, concentration of some of the phenolics, and derivatives, is reported to be enhanced during storage but remained unaffected when CO₂ was used as the storage atmosphere (Gil et al., 1997). This can partly support the present data on fresh-blended strawberry purees. On the other hand, high O₂ availability in the packages headspace is reported to be related to stronger degradation of the main phenolic acids in fresh-cut pears than under oxygen-restrictive conditions (Oms-Oliu *et al.*, 2008). The reduction of phenolic acids in fresh-cut

strawberries over storage time could be due not only to the O₂ levels inside packages, but also to their conversion to flavonoids via the action of phenylalanine ammonio lyase activity (PAL) through the phenylpropanoid metabolism (Odriozola-Serrano et al., 2010).

Total anthocyanin content mean values related to all sampling times resulted to be significantly higher in passive PP/EVOH conditions compared to all other treatments (Table 4.1). Changes over time showed a slight increase in all treatments with passive PP/EVOH MAP samples always showing the highest value although not always statistically significant. No very clear relationship could be observed in this experiment between color and TAC changes. Only at second sampling time (6 days at 5 °C) both color and TAC showed most significant differences among treatments, without noticeable connection between these quality attributes (data not shown). The increase in anthocyanin content took place during storage, especially in air where metabolism is not affected as much as in CO₂-enriched atmospheres (Gil *et al.*, 1997).

As it is shown in Table 4.1, highest antioxidant capacity was observed in passive MAP conditions, although only when packaged in PP/PA film mean value referred to all storage durations resulted statistically higher, while treatment in active PP/EVOH MAP showed the lowest value. The initial AC values for fresh strawberry purees was 188.0 mg TE 100 g⁻¹ FW. Throughout storage passive MAP samples always showed higher AC than those in active MAP, regardless of the packaging material. At the end of the storage time however differences among the treatments were not significant (Figure 4.5 B). In general, high antioxidant activities were maintained during the storage time. This probably can be attributed to the high content of TPC and DHA as both these components

are known to contribute to the antioxidant capacity. Similarly, Zheng et al. (2007) did not observe significant differences in AC values, total phenolics, total anthocyanins, or the individual phenolic compounds among high O₂ and air-stored fruits after 14 days of storage, despite the role of TPC and vitamin C as antioxidant properties.

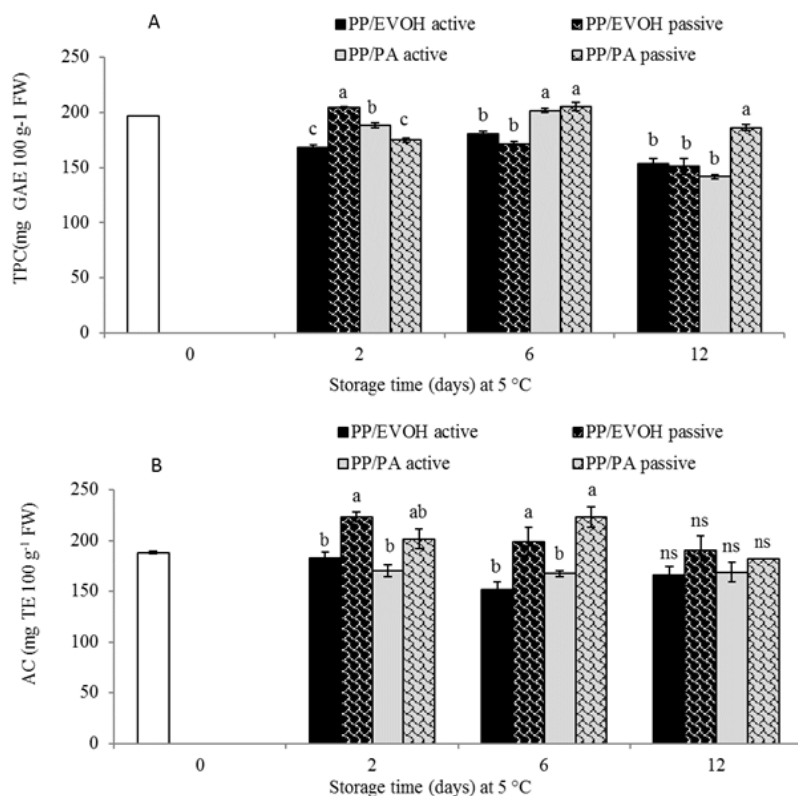


Figure 4.5 Effect of packaging condition (PP/EVOH and PP/PA active (5% O₂+13% CO₂) and passive (air) on (A) total phenolic content (TPC), (B) antioxidant capacity (AC) of fresh-blended ‘Festival’ strawberry purees stored for 12 days at 5°C mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey’ s test (*P* value≤0.05) and ns = no significance.

Microbiological analysis

According to the specifications proposed by Regulation E.C. (No. 2073/2005), at the end of the shelf life the total aerobic mesophilic bacteria should not exceed 6 log CFU g⁻¹ (Nogales-Delgado *et al.*, 2013). As reported in Table 4.1, treatment, storage and their interaction significantly ($P < 0.001$) influenced the assessed microbiological load. In general, samples stored in active PP/EVOH MAP conditions showed the lowest count mean values referred to all sampling times, for all type of assayed microorganisms. On the other hand, samples in passive PP/PA conditions always showed the highest mean values.

Evolution over time of microbial counts is presented in Figure 4.7. After 2 days of storage, no significant difference was observed among treatments for all microbial types. After 6 days however, microbial growth slightly decreased for all the treatments, but particularly visible in the case of active PP/EVOH samples. Whereas aerobic mesophilic bacteria were significantly lower compared to the other treatments. After 12 days at 5°C, purees stored in PP/PA passive condition showed an increased growth of aerobic mesophilic bacteria, although the count was still below 5 log CFU g⁻¹ (Figure 4.6 A). Psychrophilic counts were detected below 4 log CFU g⁻¹ throughout the first 6 days of storage. An increase in this bacterial group count was observed at the end of storage time, with samples packaged under passive conditions showing significantly higher psychrophilic loads, if compared with treatments under active MAP (Figure 4.6 B). Similarly, yeast and molds counts were not different among the treatments during the first 6 days while at the end of storage PP/PA passive samples showed significantly highest contamination compared to all other treatments (Figure 4.6 C). Among tested

packaging conditions (plastic materials and initial gas concentrations), the active PP/EVOH MAP seems to significantly inhibit the growth of aerobic mesophilic bacteria and yeast and molds. Although there was no significant difference in the CO₂ concentration at end of the storage, its inhibiting effect may have delayed further growth in the active MAP samples. It has also been reported that CO₂-enriched atmospheres can be beneficial in delaying microbial growth on some fresh-cut fruits and vegetables (Zhang and Watkins, 1998; Kader, 2002b).

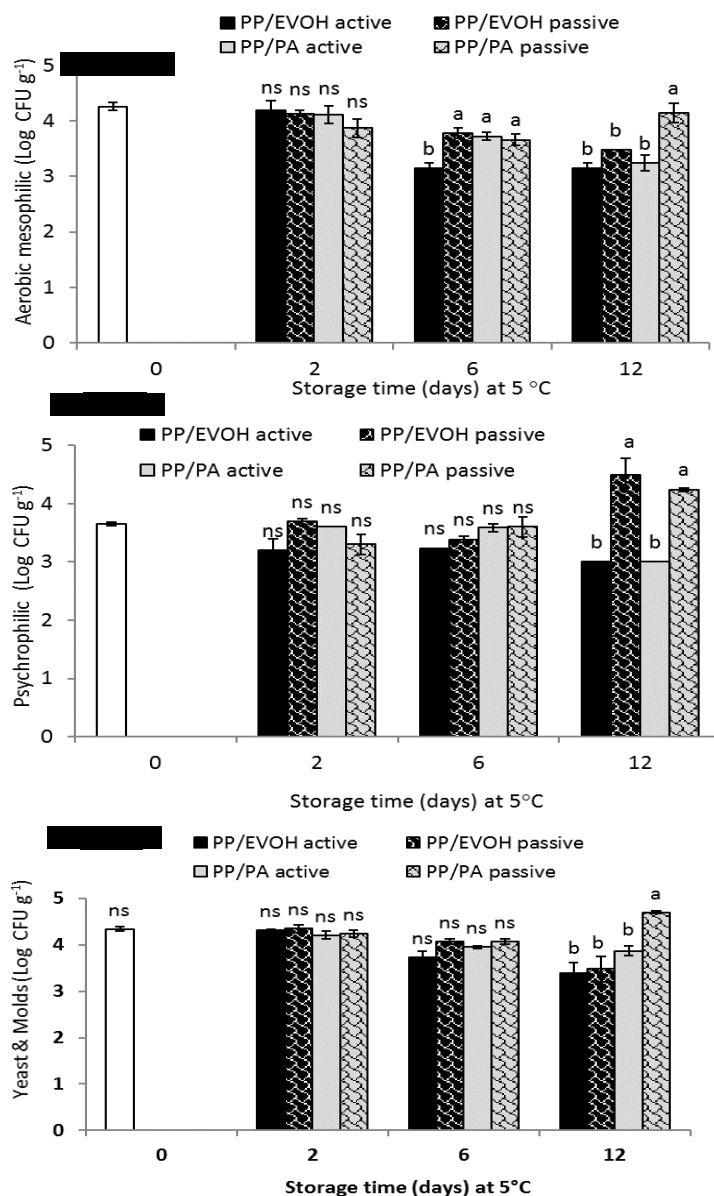


Figure 4.6 Effect of packaging condition (PP/EVOH or PP/PA) active and passive MAP on spoilage microbial counts (A) aerobic mesophilic, (B) psychrophilic, and (C) yeast & molds of strawberry purees stored for 12 days at 5°C; mean values (n=3) and standard errors bars. Means with different letters at the same storage time are significantly different according to Tukey's test (P value ≤ 0.05) and ns = no significance.

Conclusions

The results of the present study revealed that packaging conditions, storage time and their interaction significantly affected several physicochemical, nutritional quality attributes and microbiological counts of fresh-blended 'Festival' strawberry puree. However, while the tested films did not show large differences in the evolution of gas concentrations within the package in both passive and active conditions, it was possible to observe a beneficial effect of active MAP (5% O₂+13% CO₂) using both PP/EVOH and PP/PA. This definitely had an influence on the quality attributes such as color, ascorbic acid and phenols and microbiological counts. The adopted active MAP conditions contributed to maintain gas levels within the target ranges only for oxygen, while CO₂ levels tended to decrease and stabilize at about one half of the target. Anyhow, when oxygen concentration was maintained low and carbon dioxide was higher than in air effects on quality and microbial growth of fresh-blended strawberry purees could be observed.

These packaging conditions can therefore be used to store fresh-blended strawberry purees at 5°C minimizing losses of physicochemical, sensory and nutritional attributes, showing an inhibition of microbial growth compared to the passive MAP. Among the tested packaging materials PP/EVOH showed some advantages over PP/PA as it resulted in significantly lower microbial counts and no off-flavor development after 12 days of storage. Active MAP in PP/PEVOH can be applied to provide convenience to consumers and extend shelf life of fresh-blended strawberry purees without any chemical or thermal pretreatment of the product.

PART THREE: GENERAL CONCLUSIONS

General Conclusions

Strawberry as a fruit and its value added products are highly demanded in the market with consumer acceptance based on many factors of fundamental significance including color, flavor, nutritional content, organoleptic properties, antioxidant capacity etc. Fresh strawberry purees are one of the products that can be introduced as a ready- to- use item into the market with expectations of positive acceptance provided that nutritional, organoleptic and technological characteristics are suitably maintained. For the maintenance of optimum nutritional, organoleptic and technological aspects of fresh purees, they need to be minimally processed. Therefore, it is important to identify the factors that contribute towards the degradation of the nutritional and organoleptic attributes of added value food products prepared from strawberries.

However, quality changes and its maintenance upon processing remained to be critical for ready-to-use fresh strawberry puree. Because most of the quality attributes such as color and vitamin C content start to change soon after blending. Furthermore, several others are prone to changes over time although can be influenced by several factors among which are the degree of damage during processing, cultivar, processing factors (time and temperature) and storage and packaging conditions, as well as their related changes in respiration rate and other physiological responses.

Results of the present work revealed that critical aspects related to the development of fresh strawberry puree with high nutritional values, appealing organoleptic characteristic and suitable from technological point of view, with no chemical or thermal treatments were addressed. New and more convenience fresh puree was successfully developed from fresh strawberry fruits.

Based on this study, effects of increased respiration rate consequential from cell disintegration of wounded tissue up to certain wounding degree (64 pieces cutting), could be minimized significantly at a higher wounding intensity (cutting into 128 pieces and chopped) in strawberry fruits. This resulted in significant increase in total phenolic content, dehydroascorbic acid, antioxidant capacity during storage time with no effects on total anthocyanin content, total phenolic content, pH value and sugar/acid ratio.

The quality of fresh strawberry puree as an emerging high convenience products like other fresh products is influenced by cultivar variation. Among the most important cultivars grown in Italy and surveyed for suitability in puree, 'Festival' followed by 'San Andreas' had better color stability (lower total color difference (ΔE^*), higher total anthocyanin content), while 'Candonga' had superior total phenolic content and antioxidant capacity besides other quality attributes assessed and hence proved to be appropriate for fresh-blended strawberry purees.

In the minimal processing of strawberry purees, blending temperature significantly affected color, vitamin C, total anthocyanin contents and sensory attributes. Blending at 5°C resulted in better color retention (lower ΔE^*) and higher vitamin C content, aroma with a corresponding high overall rating scores after 8 days of storage at 5°C compared to blending at 21°C, though both had the same blending time of 90 s. Suggesting a blending time of 90 s at lower temperature (5°C) could be optimum for fresh quality strawberry purees. Modified atmosphere packaging (MAP) is a well-known technology used to enhance the shelf-life of fresh product during distribution the sales, through reduction respiration rate and inhibition of microbial growth. However, this technique is

effective when the composition of low oxygen and high carbon dioxide in packaging are well controlled or balanced. It was found that, active MAP (5% O₂ +13% CO₂) using PP/EVOH and PP/PA was better than passive MAP in the same materials for the storage of strawberry purees. They provided fresh-blended strawberry purees at 5°C without significant losses of physicochemical, sensory and nutritional attributes, showing an inhibition of microbial growth for 6 days. But active MAP in PP/EVOH had superior characteristics than active MAP in PP/PA due to its ability to significantly lower microbial counts with no off-flavor development even after 12 days of storage. Accordingly, for use in industry, active MAP in PP/EVOH could be recommended to provide convenience to consumers and extend shelf life of fresh-blended strawberry purees without any chemical or thermal pretreatment.

As a final remark, high convenience fresh-blended strawberry puree can successfully be developed without significant losses of nutritional, organoleptic attributes for ready-to-use with 100% fruit product puree. The influences of pre-harvest factors on quality and flavor profiles and non-thermal processing techniques should be considered for future studies.

Acknowledgements

This work was part of the project 'OFRALSER' - High Convenience Fruits and Vegetables: New Technologies for Quality and New Products' (PON01-01435), funded by 'Ministero dell'Istruzione, dell'Università e della Ricerca' - Programma Operativo Nazionale Ricerca e competitività 2007-2013.

References

- Aaby K., Mazur S., Nes A. and Skrede G. (2012). Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chem*, 132(1), 86-97.
- Adams J. B. and Brown H. M. (2007). Discoloration in raw and processed fruits and vegetables. *Crit Rev Food Sci Nutr*, 47(3), 319-333.
- Aday M. S. and Caner C. (2014). Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. *LWT - Food Science and Technology*, 57(1), 344-351.
- Agar I. T., Massantini R., Hess-Pierce B. and Kader A. A. (1999). Postharvest CO₂ and ethylene production and quality maintenance of fresh-cut kiwifruit slices. *J Food Sci*, 64(3), 433-440.
- Aguilo-Aguayo I., Soliva-Fortuny R. and Martin-Belloso O. (2009). Changes in viscosity and pectolytic enzymes of tomato and strawberry juices processed by high-intensity pulsed electric fields. *International Journal of Food Science and Technology*, 44(11), 2268-2277.
- Ahmed J., Shivhare U. S. and Raghavan G. S. V. (2004). Thermal degradation kinetics of anthocyanin and visual colour of plum puree. *European Food Research and Technology*, 218(6), 525-528.
- Al-Maiman S. A. (1997). Quality of stored strawberry puree produced in an oxygen-reduced environment.
- Aliasgarian S., Ghassemzadeh Hamid R., Moghaddam M. and Ghaffari H. (2015). Mechanical Damage Of Strawberry During Harvest And Postharvest Operations. *Acta Technologica Agriculturae*. 18: 1.
- Álvarez-Fernández M. A., Hornedo-Ortega R., Cerezo A. B., Troncoso A. M. and García-Parrilla M. C. (2014). Effects of the strawberry (*Fragaria ananassa*) purée elaboration process on non-anthocyanin phenolic composition and antioxidant activity. *Food Chem*, 164, 104-112.
- Alvarez-Suarez J. M., Giampieri F., Tulipani S., Casoli T., Di Stefano G., Gonzalez-Paramas A. M., Santos-Buelga C., Busco F., Quiles J. L., Cordero M. D., Bompadre S., Mezzetti B. and Battino M. (2014). One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *J Nutr Biochem*, 25(3), 289-294.

- Amanatidou A., Slump R., Gorris L. and Smid E. (2000). High Oxygen and High Carbon Dioxide Modified Atmospheres for Shelf-life Extension of Minimally Processed Carrots. *J Food Sci*, 65(1), 61-66.
- Arendse E., Fawole O. A. and Opara U. L. (2014). Effects of postharvest storage conditions on phytochemical and radical-scavenging activity of pomegranate fruit (cv. Wonderful). *Scientia Horticulturae*, 169, 125-129.
- Artés F., Gómez P. A. and Artés-Hernández F. (2007). Physical, Physiological and Microbial Deterioration of Minimally Fresh Processed Fruits and Vegetables. *Food Science and Technology International*, 13(3), 177-188.
- Ayala-Zavala J. F., Wang S. Y., Wang C. Y. and Gonzalez-Aguilar G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 37(7), 687-695.
- Baş D. and Boyacı İ. H. (2007). Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering*, 78(3), 836-845.
- Becerra-Moreno A., Redondo-Gil M., Benavides J., Nair V., Cisneros-Zevallos L. and Jacobo-Velazquez D. A. (2015). Combined effect of water loss and wounding stress on gene activation of metabolic pathways associated with phenolic biosynthesis in carrot. *Front Plant Sci*, 6, 837.
- Bermejo-Prada A., Segovia-Bravo K. A., Guignon B. and Otero L. (2015). Effect of hyperbaric storage at room temperature on pectin methylesterase activity and serum viscosity of strawberry juice. *Innovative Food Science & Emerging Technologies*, 30, 170-176.
- Bezerra M. A., Santelli R. E., Oliveira E. P., Villar L. S. and Escalera L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, 76(5), 965-977.
- Bhat R. and Stamminger R. (2015). Impact of ultraviolet radiation treatments on the physicochemical properties, antioxidants, enzyme activity and microbial load in freshly prepared hand pressed strawberry juice. *Food Sci Technol Int*, 21(5), 354-363.
- Boča S., Galoburda R., Skrupskis I. and Skrupska D. (2014). The Effect of Freezing and Hydrocolloids on the Physical Parameters of Strawberry Mass-Based Desserts. *Proceedings of the Latvia University of Agriculture*. 31: 12.

- Bodelón O. G., Avizcuri J.-M., Fernández-Zurbano P., Dizy M. and Préstamo G. (2013). Pressurization and cold storage of strawberry purée: Colour, anthocyanins, ascorbic acid and pectin methylesterase. *LWT - Food Science and Technology*, 52(2), 123-130.
- Brand-Williams W., Cuvelier M. E. and Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25-30.
- Brecht J. K. (1995). Physiology of Lightly Processed Fruits and Vegetables. *Hortscience*, 30(1), 18-22.
- Brunner T. A., van der Horst K. and Siegrist M. (2010). Convenience food products. Drivers for consumption. *Appetite*, 55(3), 498-506.
- Buckley M., Cowan C. and McCarthy M. (2007). The convenience food market in Great Britain: Convenience food lifestyle (CFL) segments. *Appetite*, 49(3), 600-617.
- Bursac Kovacevic D., Putnik P., Dragovic-Uzelac V., Vahcic N., Babojelic M. S. and Levaj B. (2015). Influences of organically and conventionally grown strawberry cultivars on anthocyanins content and color in purees and low-sugar jams. *Food Chem*, 181, 94-100.
- Cabezas-Serrano A. B., Amodio M. L., Cornacchia R., Rinaldi R. and Colelli G. (2009). Screening quality and browning susceptibility of five artichoke cultivars for fresh-cut processing. *J Sci Food Agric*, 89(15), 2588-2594.
- Caleb O. J., Mahajan P. V., Al-Said F. A. and Opara U. L. (2013). Modified Atmosphere Packaging Technology of Fresh and Fresh-cut Produce and the Microbial Consequences-A Review. *Food and Bioprocess Technology*, 6(2), 303-329.
- Caleb O. J., Mahajan P. V., Opara U. L. and Witthuhn C. R. (2012a). Modelling the respiration rates of pomegranate fruit and arils. *Postharvest Biology and Technology*, 64(1), 49-54.
- Caleb O. J., Opara U. L. and Witthuhn C. R. (2012b). Modified Atmosphere Packaging of Pomegranate Fruit and Arils: A Review. *Food and Bioprocess Technology*, 5(1), 15-30.
- Caner C., Aday M. S. and Demir M. (2008). Extending the quality of fresh strawberries by equilibrium modified atmosphere packaging. *European Food Research and Technology*, 227(6), 1575-1583.

- Cao X., Bi X., Huang W., Wu J., Hu X. and Liao X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science & Emerging Technologies*, 16, 181-190.
- Castaigne F., Toupin C. and Desilets D. (1991). Mathematical modeling of gas exchange in modified atmosphere packaging. *Transactions of the Asae*, 34(1), 239-0245.
- Chung T. T., West G. and Tucker G. A. (2006). Effect of wounding on cell wall hydrolase activity in tomato fruit. *Postharvest Biology and Technology*, 40(3), 250-255.
- Colantuono F., Piazzolla F., de Chiara M. L. V., Amodio M. L. and Colelli G. (2015a). *INFLUENCE OF TEMPERATURE AND BLENDING TIME ON QUALITY OF MINIMALLY PROCESSED PUREE FROM FOUR MELON TYPES*. Paper presented at the VI International Conference on Managing Quality in Chains. <http://dx.doi.org/10.17660/ActaHortic.2015.1091.19>
- Colelli G. and Martelli S. (1995). Beneficial effects on the application of CO₂-enriched atmospheres on fresh strawberries (*Fragaria X ananassa* Duch.). *Advances in Horticultural Science*, 9(2), 55-60.
- Corbo M., Campaniello D., Speranza B., Bevilacqua A. and Sinigaglia M. (2015). Non-Conventional Tools to Preserve and Prolong the Quality of Minimally-Processed Fruits and Vegetables. *Coatings*, 5(4), 931.
- Cordenunsi B. R., do Nascimento J. R. O., Genovese M. I. and Lajolo F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *J Agric Food Chem*, 50(9), 2581-2586.
- Cordenunsi B. R., Genovese M. I., Oliveira do Nascimento J. R., Aymoto Hassimotto N. M., José dos Santos R. and Lajolo F. M. (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chem*, 91(1), 113-121.
- Cordenunsi B. R., Nascimento J. R. O. and Lajolo F. M. (2003). Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chem*, 83(2), 167-173.
- Cornacchia R., Cabezas-Serrano A., Amodio M. and Colelli G. (2011). Suitability of 4 Potato Cultivars (*Solanum tuberosum* L.) to be Processed as Fresh-Cut Product. Early Cultivars. *American Journal of Potato Research*, 88(5), 403-412.
- Costa A. I. d. A., Schoolmeester D., Dekker M. and Jongen W. M. F. (2007). To cook or not to cook: A means-end study of motives for choice of meal solutions. *Food Quality and Preference*, 18(1), 77-88.

- Costa F. B., Duarte P. S., Puschmann R. and Finger F. L. (2011). Quality of fresh-cut strawberry. *Horticultura Brasileira*, 29, 477-484.
- Costell E., Carbonell I., Tàrrega A., Bayarri S. and Kilcast D. (2010). Sensory quality control of fresh produce. *Sensory analysis for food and beverage quality control*. Woodhead, Cambridge, 276-292.
- Daniels S., Glorieux I., Minnen J., van Tienoven T. P. and Weenas D. (2015). Convenience on the menu? A typological conceptualization of family food expenditures and food-related time patterns. *Social Science Research*, 51(0), 205-218.
- Davey M. W., Montagu M. V., Inzé D., Sanmartin M., Kanellis A., Smirnoff N., Benzie I. J. J., Strain J. J., Favell D. and Fletcher J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric*, 80(7), 825-860.
- Dubrovi I., Herceg Z., Jambrak A. R. e., Badanjak M. and Dragovi-Uzelac V. (2011). Effect of High Intensity Ultrasound and Pasteurization on Anthocyanin Content in Strawberry Juice. *Food Technol. Biotechnol.*, 49(2), 196–204.
- El-Ramady H., Domokos-Szabolcsy É., Abdalla N., Taha H. and Fári M. (2015). Postharvest Management of Fruits and Vegetables Storage. In Lichtfouse E. (Ed.), *Sustainable Agriculture Reviews* (Vol. 15, pp. 65-152): Springer International Publishing.
- Erkan M., Wang S. Y. and Wang C. Y. (2008). Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit. *Postharvest Biology and Technology*, 48(2), 163-171.
- FAOSTAT. (2015, September). Food and Agriculture Organization of the United Nations (FOA) data,.Available on [<http://top5ofanything.com/list/35769269/Strawberry-Producing-Countries>], last accessed by the top 5 of everthing September 2015.
- Fernandes V. C., Domingues V. F., Mateus N. and Delerue-Matos C. (2012). Analysing organochlorine pesticides in strawberry jams using GC-ECD, GC-MS/MS and QuEChERS sample preparation. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 29(7), 1074-1084.
- Fernández-Lara R., Gordillo B., Rodríguez-Pulido F. J., Lourdes González-Miret M., del Villar-Martínez A. A., Dávila-Ortiz G. and Heredia F. J. (2015). Assessment of the differences in the phenolic composition and color characteristics of new strawberry (*Fragaria x ananassa* Duch.) cultivars by HPLC–MS and Imaging Tristimulus Colorimetry. *Food Research International*, 76, Part 3, 645-653.

- Fernández-León M. F., Fernández-León A. M., Lozano M., Ayuso M. C., Amodio M. L., Colelli G. and González-Gómez D. (2013). Retention of quality and functional values of broccoli 'Parthenon' stored in modified atmosphere packaging. *Food Control*, 31(2), 302-313.
- Francis G. A., Gallone A., Nychas G. J., Sofos J. N., Colelli G., Amodio M. L. and Spano G. (2012). Factors affecting quality and safety of fresh-cut produce. *Crit Rev Food Sci Nutr*, 52(7), 595-610.
- Freedman M. (2011). Understanding convenience store shoppers: Available on:[http://www.igd.com/Understanding_convenience_store_shoppers_article], last accessed on November, 2015).
- Fu L., Xu B.-T., Xu X.-R., Gan R.-Y., Zhang Y., Xia E.-Q. and Li H.-B. (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chem*, 129(2), 345-350.
- Giampieri F., Alvarez-Suarez J. M. and Battino M. (2014). Strawberry and Human Health: Effects beyond Antioxidant Activity. *J Agric Food Chem*, 62(18), 3867-3876.
- Giampieri F., Tulipani S., Alvarez-Suarez J. M., Quiles J. L., Mezzetti B. and Battino M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, 28(1), 9-19.
- Gil M. I., Holcroft D. M. and Kader A. A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J Agric Food Chem*, 45(5), 1662-1667.
- Giuggioli N. R., Girgenti V., Baudino C. and Peano C. (2015). Influence of Modified Atmosphere Packaging Storage on Postharvest Quality and Aroma Compounds of Strawberry Fruits in a Short Distribution Chain. *Journal of Food Processing and Preservation*, 39(6), 3154-3164.
- Gol N. B., Patel P. R. and Rao T. V. R. (2013). Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. *Postharvest Biology and Technology*, 85, 185-195.
- Gössinger M., Mayer F., Radocha N., Höfler M., Boner A., Groll E., Nosko E., Bauer R. and Berghofer E. (2009). Consumer's color acceptance of strawberry nectars from puree. *Journal of Sensory Studies*, 24(1), 78-92.
- Gündüz K. and Özdemir E. (2014). The effects of genotype and growing conditions on antioxidant capacity, phenolic compounds, organic acid and individual sugars of strawberry. *Food Chem*, 155, 298-303.

- Gunes G. and Lee C. Y. (1997). Color of Minimally Processed Potatoes as Affected by Modified Atmosphere Packaging and Antibrowning Agents. *J Food Sci*, 62(3), 572-575.
- Henriqueta T. G. B., Alexandra M. G. N. M., Antonio G. S., Luiz C. d. C. M. and Elen V. P. (2014). Postharvest technologies for fresh and minimally processed strawberry conservation In Nathan Molane (Ed.), *Strawberres - Cultivation, antioxidant property and health benefits*. New York, USA: Nova.
- Hodges D. M. and Toivonen P. M. A. (2008). Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biology and Technology*, 48(2), 155-162.
- Holcroft D. M. and Kader A. A. (1999). Carbon Dioxide-induced Changes in Color and Anthocyanin Synthesis of Stored Strawberry Fruit. *Hortscience*, 34(7), 1244-1248.
- Hussein Z., Caleb O. J., Jacobs K., Manley M. and Opara U. L. (2015). Effect of perforation-mediated modified atmosphere packaging and storage duration on physicochemical properties and microbial quality of fresh minimally processed 'Acco' pomegranate arils. *LWT - Food Science and Technology*, 64(2), 911-918.
- Imahori Y., Zhou Y.-F., Ueda Y., Abe K. and Chachin K. (1997). Effects of Wound Stress by Slicing Sweet Pepper Fruits on Ascorbic Acid Metabolism. *Journal of the Japanese Society for Horticultural Science*, 66(1), 175-183.
- Italiafruit News. (2016). Report fragola: varietà, produzione, consumi, prezzi, import-export. Available from: <http://www.italiafruit.net/DettaglioNews/34040/la-categoria-del-mese/report-fragola-varietà-produzione-consumi-prezzi-import-export> (Last accessed on 28-03-2016).
- Jouki M. and Khazaei N. (2014). Effect of low-dose gamma radiation and active equilibrium modified atmosphere packaging on shelf life extension of fresh strawberry fruits. *Food Packaging and Shelf Life*, 1(1), 49-55.
- Jouquand C., Chandler C., Plott A. and Goodner K. (2008). A Sensory and Chemical Analysis of Fresh Strawberries Over Harvest Dates and Seasons Reveals Factors That Affect Eating Quality. *J. AMER. Soc. HORT. Sci.*, 133(6), 859-867.
- Kader A. A. (1991). Quality and its maintenance in relation to the postharvest physiology of strawberry. In A. Dale and J.J. Luby (Eds.), *The strawberry into the 21 : Timber Press, Portland, Oregon*.

- Kader A. A. (Ed.) (2002a). *Methods of gas mixing, sampling and analysis* (Vol. Pub. 3311). Oakland, CA: University of California.
- Kader A. A. (2002b). Quality parameters of fresh-cut fruit and vegetable products. *Fresh-cut fruits and vegetables*, 11-20.
- Kader A. A. and Saltveit M. E. (Eds.). (2003). *Respiration and gas exchange*. New York, NY: Marcel Dekker Inc.
- Kafkas E., Koşar M., Paydaş S., Kafkas S. and Başer K. H. C. (2007). Quality characteristics of strawberry genotypes at different maturation stages. *Food Chem*, 100(3), 1229-1236.
- Kalt W., Forney C. F., Martin A. and Prior R. L. (1999). Antioxidant Capacity, Vitamin C, Phenolics, and Anthocyanins after Fresh Storage of Small Fruits. *J Agric Food Chem*, 47(11), 4638-4644.
- Kanellis A. and Solomos T. (1985). *The effect of low oxygen on the activities of pectinmethylesterase and acid phosphatase during the course of ripening of bananas*. Paper presented at the Proc. 4th Nat. CA Res. Conf., Hort. Rpt.
- Kang H. M. and Saltveit M. E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *J Agric Food Chem*, 50(26), 7536-7541.
- Khuri A. I. (2006). Response Surface Methodology And Related Topics. Chapter 4: Statistical inference for response surface optima. Lin D. K. J. and Peterson J. J. New Jersey World Scietific.
- Kiran B., Pathak K., Kumar R. and Deshmukh D. (2016). Statistical optimization using Central Composite Design for biomass and lipid productivity of microalga: A step towards enhanced biodiesel production. *Ecological Engineering*, 92, 73-81.
- Lafta A. M. and Fugate K. K. (2011). Metabolic profile of wound-induced changes in primary carbon metabolism in sugarbeet root. *Phytochemistry*, 72(6), 476-489.
- Lagarón J. M. (2011). Multifunctional and nanoreinforced polymers for food packaging *Multifunctional and Nanoreinforced Polymers for Food Packaging* (pp. 1-28).
- Lamikanra O. (2002). Fresh-cut Fruits and Vegetables: science, technology and market. Lamikanra O. New York,USA, CRC PRESS.
- Lee L., Arul J., Lencki R. and Castaigne F. (1996). A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: Physiological basis and practical aspects—part II. *Packaging Technology and Science*, 9(1), 1-17.

- Lee S. K. and Kader A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 20(3), 207-220.
- Lester G. E. (2003). *Oxidative stress affecting fruit senescence*. Paper presented at the Postharvest Oxidative Stress in Horticultural Crops, Binghamton, New York.
- Lin L.-S. and Varner J. E. (1991). Expression of ascorbic acid oxidase in zucchini squash (*Cucurbita pepo* L.). *Plant Physiol*, 96(1), 159-165.
- Livinali E., Sperotto R. A., Ferla N. J. and de Souza C. F. V. (2014). Physicochemical and nutritional alterations induced by two-spotted spider mite infestation on strawberry plants. *Electronic Journal of Biotechnology*, 17(5), 193-198.
- Loewus F. A., Jang R., Seegmiller C. G. and With the technical assistance of Walter Mann J. (1958). THE CONVERSION OF C14-LABELED SUGARS TO L-ASCORBIC ACID IN RIPENING STRAWBERRIES : IV. A COMPARATIVE STUDY OF D-GALACTURONIC ACID AND L-ASCORBIC ACID FORMATION. *Journal of Biological Chemistry*, 232(1), 533-541.
- Maceiras R., Álvarez E. and Cancela M. A. (2007). Rheological properties of fruit purees: Effect of cooking. *Journal of Food Engineering*, 80(3), 763-769.
- Mahajan P. V., Caleb O. J., Singh Z., Watkins C. B. and Geyer M. (2014). Postharvest treatments of fresh produce. *Philosophical transactions. Series A, Mathematical, physical, and engineering sciences*, 372(2017), 20130309.
- Mahmood T., Anwar F., Abbas M., Boyce M. C. and Saari N. (2012). Compositional Variation in Sugars and Organic Acids at Different Maturity Stages in Selected Small Fruits from Pakistan. *Int J Mol Sci*, 13(2), 1380-1392.
- Marketsandmarkets (2016). FRUIT & VEGETABLE PROCESSING MARKET BY PRODUCT TYPE (FRESH, FRESH CUT, CANNED, FROZEN, DRIED, CONVENIENCE), EQUIPMENT (PRE-PROCESSING, PROCESSING, WASHING & DEWATERING, FILLING, SEASONING, PACKAGING), OPERATION & REGION GLOBAL TREND & FORECAST TO 2019
- Marsh K., Attanayake S., Walker S., Gunson A., Boldingh H. and MacRae E. (2004). Acidity and taste in kiwifruit. *Postharvest Biology and Technology*, 32(2), 159-168.
- Marszalek K., Mitek M. and Skapska S. (2015). The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical

composition, microbial and enzyme activity in strawberry puree. *Innovative Food Science & Emerging Technologies*, 27, 48-56.

Martín-Belloso O. and Soliva-Fortuny R. (2011). *Advances in Fresh-Cut Fruits and Vegetables Processing*. New York, USA: CRC Press-Taylor & Francis.

Martínez-Romero D., Serrano M., Carbonell A., Castillo S., Riquelme F. and Valero D. (2004). Mechanical Damage During Fruit Post-Harvest Handling: Technical and Physiological Implications. In Dris R. and Jain S. M. (Eds.), *Production Practices and Quality Assessment of Food Crops: Quality Handling and Evaluation* (pp. 233-252). Dordrecht: Springer Netherlands.

Martínez I., Ares G. and Lema P. (2008). Influence of cut and packaging film on sensory quality of fresh-cut butterhead lettuce (*Lactuca sativa* L., CV. Wang). *Journal of Food Quality*, 31(1), 48-66.

McIntosh W. A. (1996). *Sociologies of food and nutrition*. New York, NY: Plenum Press.

Mehta R. A., Parsons B. L., Mehta A. M., Nakhasi H. L. and Mattoo A. K. (1991). Differential protein metabolism and gene expression in tomato fruit during wounding stress. *Plant and Cell Physiology*, 32(7), 1057-1065.

Mena P., García-Viguera C., Navarro-Rico J., Moreno D. A., Bartual J., Saura D. and Martí N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *J Sci Food Agric*, 91(10), 1893-1906.

Merchante C., Vallarino J. G., Osorio S., Aragüez I., Villarreal N., Ariza M. T., Martínez G. A., Medina-Escobar N., Civello M. P., Fernie A. R., Botella M. A. and Valpuesta V. (2013). Ethylene is involved in strawberry fruit ripening in an organ-specific manner. *J Exp Bot*, 64(14), 4421-4439.

Meyers K. J., Watkins C. B., Pritts M. P. and Liu R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *J Agric Food Chem*, 51(23), 6887-6892.

Mitcham E. J., C.H. Crisosto and Kader. A. A. (2000). Strawberry: Recommendations for maintaining postharvest quality. Retrieved from <http://postharvest.ucdavis.edu/PFfruits/Strawberry/>

Morales-Soto A., García-Salas P., Rodríguez-Pérez C., Jiménez-Sánchez C., Cádiz-Gurrea M. d. I. L., Segura-Carretero A. and Fernández-Gutiérrez A. (2014). Antioxidant capacity of 44 cultivars of fruits and vegetables grown in Andalusia (Spain). *Food Research International*, 58(0), 35-46.

- Moreira M. R., Tomadoni B., Martín-Belloso O. and Soliva-Fortuny R. (2015). Preservation of fresh-cut apple quality attributes by pulsed light in combination with gellan gum-based prebiotic edible coatings. *LWT - Food Science and Technology*, 64(2), 1130-1137.
- Nielsen G. S. (2016). IV gamma, dal 2015 un'ulteriore spinta alla crescita del mercato nazionale. *Olioofficina Magazine*, Nielsen HHP Trade Planner - Totale Italia - Dicembre 2015.
- Nielsen T. and Leufven A. (2008). The effect of modified atmosphere packaging on the quality of Honeoye and Korona strawberries. *Food Chem*, 107(3), 1053-1063.
- Nilsson F. and Hedenqvist M. S. (2011). Mass transport and high barrier properties of food packaging polymers *Multifunctional and Nanoreinforced Polymers for Food Packaging* (pp. 129-151).
- Nishiyama I., Fukuda T., Shimohashi A. and Oota T. (2008). Sugar and Organic Acid Composition in the Fruit Juice of Different *Actinidia* Varieties. *Food Science and Technology Research*, 14(1), 67-73.
- Nogales-Delgado S., Fernandez-Leon A. M., Delgado-Adamez J., Hernandez-Mendez M. T. and Bohoyo-Gil D. (2013). Effects of Several sanitisers for Improving quality attributes of minimally processed strawberry *Fragaria vesca*. *Czech journal of food sciences*, 31, 49-54.
- Nunes M., Morais A., Brecht J. and Sargent S. (1995). *Quality of strawberries after storage in controlled atmospheres at above optimum storage temperatures*. Paper presented at the PROCEEDINGS-FLORIDA STATE HORTICULTURAL SOCIETY.
- Odriozola-Serrano I., Soliva-Fortuny R. and Martín-Belloso O. (2010). Changes in bioactive composition of fresh-cut strawberries stored under superatmospheric oxygen, low-oxygen or passive atmospheres. *Journal of Food Composition and Analysis*, 23(1), 37-43.
- Olsson M. E., Ekvall J., Gustavsson K. E., Nilsson J., Pillai D., Sjöholm I., Svensson U., Akesson B. and Nyman M. G. (2004). Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria x ananassa*): effects of cultivar, ripening, and storage. *J Agric Food Chem*, 52(9), 2490-2498.
- Oms-Oliu G., Odriozola-Serrano I. and Martín-Belloso O. (2012). *The effects of non-thermal technologies on phytochemicals*: INTECH Open Access Publisher.

- Oms-Oliu G., Odrjzola-Serrano I., Soliva-Fortuny R. and Martín-Belloso O. (2008). Antioxidant content of fresh-cut pears stored in high-O₂ active packages compared with conventional low-O₂ active and passive modified atmosphere packaging. *J Agric Food Chem*, 56(3), 932-940.
- Oms-Oliu G. and Soliva-Fortuny R. (2011). 15 Future Trends in Fresh-Cut Fruit and Vegetable Processing. In Martín-Belloso O. and Soliva-Fortuny R. (Eds.), *Advances in Fresh-Cut Fruits and Vegetables Processing* (pp. 377-386). New York, USA: CRC Press, Taylor and Francis Group, LLC
- Padmanabhan P., Mizran A., Sullivan J. A. and Paliyath G. (2016). Strawberries. In Caballero B., Finglas P. M. and Toldrá F. (Eds.), *Encyclopedia of Food and Health* (pp. 193-198). Oxford: Academic Press.
- Parsons Harriet T., Yasmin T. and Fry Stephen C. (2011). Alternative pathways of dehydroascorbic acid degradation in vitro and in plant cell cultures: novel insights into vitamin C catabolism. *Biochemical Journal*, 440(3), 375-385.
- Patras A., Brunton N. P., Da Pieve S. and Butler F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, 10(3), 308-313.
- Patras A., Brunton N. P., O'Donnell C. and Tiwari B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, 21(1), 3-11.
- Pelayo-Zaldívar C., Ebeler S. E. and Kader A. A. (2005). CULTIVAR AND HARVEST DATE EFFECTS ON FLAVOR AND OTHER QUALITY ATTRIBUTES OF CALIFORNIA STRAWBERRIES. *Journal of Food Quality*, 28(1), 78-97.
- Perez A. G. and Sanz C. (2001). Effect of high-oxygen and high-carbon-dioxide atmospheres on strawberry flavor and other quality traits. *J Agric Food Chem*, 49(5), 2370-2375.
- Pincemail J., Kevers C., Tabart J., Defraigne J. O. and Dommes J. (2012). Cultivars, culture conditions, and harvest time influence phenolic and ascorbic acid contents and antioxidant capacity of strawberry (*Fragaria x ananassa*). *J Food Sci*, 77(2), C205-210.
- Pineli L. d. L. d. O., Moretti C. L., dos Santos M. S., Campos A. B., Brasileiro A. V., Córdova A. C. and Chiarello M. D. (2011). Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, 24(1), 11-16.

- Ramos B., Miller F. A., Brandão T. R. S., Teixeira P. and Silva C. L. M. (2013). Fresh fruits and vegetables—An overview on applied methodologies to improve its quality and safety. *Innovative Food Science & Emerging Technologies*, 20(0), 1-15.
- Rawson A., Patras A., Tiwari B. K., Noci F., Koutchma T. and Brunton N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Research International*, 44(7), 1875-1887.
- Renault P., Houal L., Jacquemin G. and Chambroy Y. (1994). Gas-Exchange in Modified Atmosphere Packaging .2. Experimental Results with Strawberries. *International Journal of Food Science and Technology*, 29(4), 379-394.
- Reyes F., L., Emilio Villarreal J. and Cisneros-Zevallos L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chem*, 101(3), 1254-1262.
- Reyes L. F. and Cisneros-Zevallos L. (2003). Wounding Stress Increases the Phenolic Content and Antioxidant Capacity of Purple-Flesh Potatoes (*Solanum tuberosum* L.). *J Agric Food Chem*, 51(18), 5296-5300.
- Rico D., Martin-Diana A. B., Barat J. and Barry-Ryan C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology*, 18(7), 373-386.
- Rodríguez-Delgado M.-Á., González-Hernández G., Conde-González J.-E. a. and Pérez-Trujillo J.-P. (2002). Principal component analysis of the polyphenol content in young red wines. *Food Chem*, 78(4), 523-532.
- Romanazzi G., Nigro F., Ippolito A. and Salerno M. (2001). Effect of short hypobaric treatments on postharvest rots of sweet cherries, strawberries and table grapes. *Postharvest Biology and Technology*, 22(1), 1-6.
- Saltveit M. E. (1997). *Physical and physiological changes in minimally processed fruits and vegetables Phytochemistry of fruit and vegetables*. Paper presented at the Proceedings of the phytochemical society of Europe, NY.
- Saltveit M. E. (2003). Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology*, 27(1), 3-13.
- Saltveit M. E. S. (2005). Influence of heat shocks on the kinetics of chilling-induced ion leakage from tomato pericarp discs. *Postharvest Biology and Technology*, 36(1), 87-92.

- Šamec D., Maretić M., Lugarić I., Mešić A., Salopek-Sondi B. and Duralija B. (2016). Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food Chem*, 194, 828-834.
- Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT - Food Science and Technology*, 43(3), 381-392.
- Scalzo J., Politi A., Pellegrini N., Mezzetti B. and Battino M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*, 21(2), 207-213.
- Scholliers P. (2015). Convenience foods. What, why, and when. *Appetite*, 94, 2-6.
- Shamaila M., Powrie W. and Skura B. (1992). Sensory evaluation of strawberry fruit stored under modified atmosphere packaging (MAP) by quantitative descriptive analysis. *J Food Sci*, 57(5), 1168-1184.
- Simões A. D. N., Allende A., Tudela J. A., Puschmann R. and Gil M. I. (2011). Optimum controlled atmospheres minimise respiration rate and quality losses while increase phenolic compounds of baby carrots. *LWT - Food Science and Technology*, 44(1), 277-283.
- Simpson R., Acevedo C. and Almonacid S. (2009). Mass transfer of CO₂ in MAP systems: Advances for non-respiring foods. *Journal of Food Engineering*, 92(2), 233-239.
- Singleton S. L. and Rossi J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16, 144-158.
- Soliva-Fortuny R. C., Biosca-Biosca M., Grigelmo-Miguel N. and Martin-Belloso O. (2002). Browning, polyphenol oxidase activity and headspace gas composition during storage of minimally processed pears using modified atmosphere packaging. *J Sci Food Agric*, 82(13), 1490-1496.
- Sulaiman A. and Silva F. V. M. (2013). High pressure processing, thermal processing and freezing of 'Camarosa' strawberry for the inactivation of polyphenoloxidase and control of browning. *Food Control*, 33(2), 424-428.
- Sun J., Chu Y. F., Wu X. and Liu R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *J Agric Food Chem*, 50(25), 7449-7454.
- Surjadinata B. B. and Cisneros-Zevallos L. (2003). Modeling wound-induced respiration of fresh-cut carrots (*Daucus carota* L.). *J Food Sci*, 68(9), 2735-2740.

- Szczesniak A. S. and Smith B. J. (1969). OBSERVATIONS ON STRAWBERRY TEXTURE A THREE-PRONGED APPROACH. *Journal of Texture Studies*, 1(1), 65-89.
- Terefe N. S., Kleintschek T., Gamage T., Fanning K. J., Netzel G., Versteeg C. and Netzel M. (2013). Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. *Innovative Food Science & Emerging Technologies*, 19(0), 57-65.
- Terefe N. S., Matthies K., Simons L. and Versteeg C. (2009). Combined high pressure-mild temperature processing for optimal retention of physical and nutritional quality of strawberries (*Fragaria* and *Ananassa*). *Innovative Food Science & Emerging Technologies*, 10(3), 297-307.
- Terefe N. S., Yang Y. H., Knoerzer K., Buckow R. and Versteeg C. (2010). High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science & Emerging Technologies*, 11(1), 52-60.
- Thompson A. (2010a). Modified atmosphere packaging. *Controlled atmosphere storage of fruits and vegetables* (Ed. 2), 81-115.
- Thompson A. K. (2010b). *Controlled atmosphere storage of fruits and vegetables*: CABI.
- Tiziani S. and Vodovotz Y. (2005). Rheological characterization of a novel functional food: tomato juice with soy germ. *J Agric Food Chem*, 53(18), 7267-7273.
- Torrades F. and García-Montaño J. (2014). Using central composite experimental design to optimize the degradation of real dye wastewater by Fenton and photo-Fenton reactions. *Dyes and Pigments*, 100, 184-189.
- Torres-Contreras A. M., Nair V., Cisneros-Zevallos L. and Jacobo-Velázquez D. A. (2014). Plants as biofactories: Stress-induced production of chlorogenic acid isomers in potato tubers as affected by wounding intensity and storage time. *Industrial Crops and Products*, 62, 61-66.
- Tudela J. A., Espin J. and Gil M. (2002). Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26(1), 75-84.
- Tulipani S., Marzban G., Herndl A., Laimer M., Mezzetti B. and Battino M. (2011). Influence of environmental and genetic factors on health-related compounds in strawberry. *Food Chem*, 124(3), 906-913.

- Tulipani S., Mezzetti B., Capocasa F., Bompadre S., Beekwilder J., de Vos C. H., Capanoglu E., Bovy A. and Battino M. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J Agric Food Chem*, 56(3), 696-704.
- Ubeda C., Callejón R. M., Troncoso A. M., Morales M. L. and Garcia-Parrilla M. C. (2014). Influence of the production process of strawberry industrial purees on free and glycosidically bound aroma compounds. *Innovative Food Science & Emerging Technologies*, 26, 381-388.
- Valpuesta V. and Botella M. A. (2004). Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. *Trends Plant Sci*, 9(12), 573-577.
- Van De Velde F., Tarola A., Güemes D. and Pirovani M. (2013). Bioactive Compounds and Antioxidant Capacity of Camarosa and Selva Strawberries (*Fragaria x ananassa* Duch.). *Foods*, 2(2), 120.
- Van der Steen C., Jacxsens L., Devlieghere F. and Debevere J. (2002). Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology and Technology*, 26(1), 49-58.
- Vandendriessche T., Vermeir S., Mayayo Martinez C., Hendrickx Y., Lammertyn J., Nicolai B. M. and Hertog M. L. A. T. M. (2013). Effect of ripening and inter-cultivar differences on strawberry quality. *LWT - Food Science and Technology*, 52(2), 62-70.
- Wang S. Y. and Camp M. J. (2000). Temperatures after bloom affect plant growth and fruit quality of strawberry. *Scientia Horticulturae*, 85(3), 183-199.
- Wang Z., Zhang M. and Wu Q. (2015). Effects of temperature, pH, and sunlight exposure on the color stability of strawberry juice during processing and storage. *LWT - Food Science and Technology*, 60(2, Part 2), 1174-1178.
- Watada A. E., Ko N. P. and Minott D. A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*, 9(2), 115-125.
- Watada A. E. V. and Weichmann J. (1987). *Postharvest Physiology of Vegetables* (pp. pp. 455-468). New York Marcel Dekker.
- Wolucka B. A. and Van Montagu M. (2003). GDP-mannose 3',5'-epimerase forms GDP-L-gulose, a putative intermediate for the de novo biosynthesis of vitamin C in plants. *J Biol Chem*, 278(48), 47483-47490.

- Wright K. P. and Kader A. A. (1997). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, 10(1), 39-48.
- Zapata S. and Dufour J.-P. (1992). Ascorbic, Dehydroascorbic and Isoascorbic Acid Simultaneous Determinations by Reverse Phase Ion Interaction HPLC. *J Food Sci*, 57(2), 506-511.
- Zhang J. J. and Watkins C. (1998). Molecular responses of strawberry fruit to high carbon dioxide. *Hortscience*, 33(3), 517-517.
- Zhu F., Xu J., Ke Y., Huang S., Zeng F., Luan T. and Ouyang G. (2013). Applications of in vivo and in vitro solid-phase microextraction techniques in plant analysis: A review. *Analytica Chimica Acta*, 794, 1-14.
- Zhu M., Chu C. L., Wang S. L. and Lencki R. W. (2001). Influence of Oxygen, Carbon Dioxide, and Degree of Cutting on the Respiration Rate of Rutabaga. *J Food Sci*, 66(1), 30-37.