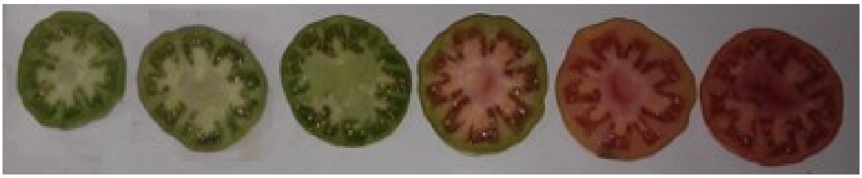
UNIVERSITÀ DI FOGGIA
Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente

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

Management of soil fertility and postharvest quality and traceability of organic horticultural products



Candidate:
Francesco Giovanni Ceglie

Tutors:
Prof. Maria Luisa Amodio

To my wife, Isabel

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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 **'Management of soil fertility and postharvest quality and traceability of organic horticultural products'**, discussed at the Università di Foggia, May V, 2016

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The organic strawberries, tomatoes and lamb's lettuce have been produced in the greenhouses of the MOREGREEN experimental device that, the Italian Ministry of Agriculure Food and Forestry Policy, has recognized as national long term experiment on organic greenhouse horticulture in the frame of the actions to support research for quality organic farming in Italy (Law n. 488/99, Art. 59 - RETIBIO program).

Management of soil fertility and postharvest quality and traceability of organic horticultural products

Contents

Extended abstract

Fruit and vegetables are characterized by strong seasonality and great perishability, therefore research and innovation in postharvest management and technology have always been at the basis of development and advances in this sector. From the production site to the consumption place, the challenge is to maintain the initial quality of the products working against the clock of the biological deterioration. Over the years, the quality of fresh fruit and vegetables on the market has been enhanced and the shelf-life has been extended. Initially, fresh fruit and vegetables were purchased according the price and their visual appearance and flavor. Meanwhile, food scandals, genetic modifications, animal abuses, label falsifications have spread out food scares among people modifying the food choice criteria. Nowadays, consumer is looking for healthy food and alternatives diets purchasing produce based on the expected nutritional proprieties, since these are barely indicated in the label (not present for spare products). The organoleptic and nutritional quality of a fresh produce on the market depends on the quality at harvest; growers are therefore called to supply extra and first quality fruits and vegetables taking care of all the factors that may improve production quality at farm level. To enhance the nutritional quality of fresh and fresh-cut products more attention should be directed towards obtaining fresh produce with superior quality in terms of vitamins, antioxidant activity and, in general, secondary metabolites. Proponents of organic products claim the superior quality of fresh fruits and vegetable produced in organic farming systems and consumer seems to trust this hypothesis. However, the scientific basis of the differences between organically and

conventionally produced fruit and vegetables are under debate, and especially the postharvest stage is slight considered in comparison studies. Recent scientific research is oriented towards developing non-destructive techniques to measure the quality and identify the origin of the products in order to support present certifications, traceability procedures and decreasing the risk of food frauds. Moreover, better forecasts of the initial quality of the product might contribute towards optimization of the postharvest management and hence to the implementation of tailored selection processes to match consumer requirements.

Organic agriculture shares 1% of total agricultural land (43.7 million hectares) by almost two and half million of operators. The organic market size of organic food and beverage, including fruits and vegetables, reached 70 billion of euro in 2014. Presently, organic standards include a well-defined set of practices and a list of technical tools that are permitted by the Regulations and that shall be complained to certify as organic any produce. Organic techniques can rely on complex agro-ecosystem management or they can just replace chemical fertilizers and pesticides with allowed inputs. Focusing to the fertility management, the fertilization influences not only crop performance and crop yields, but also crop quality (taste, firmness, internal quality, storage performance). The production techniques contribute in defining the quality characteristics of fresh fruits and vegetables at harvest but a limited amount of researches investigated whereas the production system may affect the postharvest evolution of the initial quality. Recent studies, suggest that organic farming management may create moderate stress conditions for the plants, that in response to this, accumulate sugars and secondary metabolites, such as vitamin C and phenolic compounds improving the nutritional quality of organic production. As a consequence, . these differences in composition, might determine diverse postharvest performances in respect to the different fertility management strategies. Moreover organic products may be more perishable in comparison to conventional one, as a consequence of the suffered stress and to the eventual higher microbial load, since defence methods applied for organic system may be less efficient than other chemical alternatives.

Under these hypothesis, the aim of this research was to study the quality characteristics and their evolution during storage of three target crops organically grown, and particularly, strawberries, tomatoes and lamb's lettuce. Three different organic soil fertility management where implemented under the same environmental conditions: i) a simplified organic production system based on organic commercial fertilizers (SUBST); ii) and organic production system based on cover crops and animal manure amendment (AGROMAN), and iii) and organic production system based on vegetal sources inputs like cover crops and on-farm compost (AGROCOM). Initial quality was assessed and monitored during storage, applying the recommended postharvest conditions for each species. Moreover, strawberry was used as model crop to realize a comparison study on the effect of different organic versus a conventional production system, managed in the same pedoclimatic conditions.

A second objective of this comparison was to study the potentiality of NIR spectroscopy to predict internal quality attributes and to model the differences induced by the production system to the fruits spectra in order to classify the fruits and trace the organic origin.

For organic strawberries (‘Festival’) the yields, in the were not significantly different in both years among the compared fertility management systems, while, as a whole, the yield in 2013 (30.3 Mg ha^{-1}) was significantly higher than in 2014 (28.9 Mg ha^{-1}). As for the initial quality, AGROMAN and AGROCOM allowed to obtain strawberry fruits with better characteristics in terms of color and higher phenolic content than the SUBST by the average values of the two years of investigation. During storage at 1°C (90-95% RH) for13 days, hue angle, vitamin C content and sensorial scores (sweetness and off-flavors) were significantly higher for the organic strawberry grown in SUBST compared to all other systems. In a second experiment, comparing organic and conventionally grown strawberries, conventionally grown strawberry fruits at harvest resulted higher in diameter, and firmness compared to all the organically produced strawberries. Vitamin C content was higher in SUBST than in CONV; TSS, fructose and glucose contents were higher in AGROMAN then in CONV fruits. During postharvest, initial differences in firmness, glucose and fructose and Vitamin C were maintained with also a significant accumulation of oxidized vitamin C in CONV. At the end of storage, total phenols were significantly lower in CONV than in AGROMAN and AGROCOM. In conclusion, the production system affected the quality parameters of ‘Festival’ strawberry cultivated in greenhouse under Mediterranean climate conditions. Organically grown strawberries resulted in higher nutritional compounds at harvest and maintained their initial quality better than conventional strawberries. Possible explanations for the effects of production system on nutritional quality and postharvest performance of fresh produce are considered the diverse mineralization of fertilizer and amendment supplied and the secondary metabolites accumulation as plant stress response.

In the case of tomatoes (‘Marmande’), breaker and pink tomatoes at harvest, were sampled from each system and then breaker tomatoes were subsequently stored in a cold room at 15°C and ripened up to pink stage within 10 days. The effect of the fertilization systems was studied comparing breaker tomatoes after 10 days of storage and pink tomato ripened on plant and after storage. Yield, morphological indexes, dry matter, firmness, and composition were evaluated. The three systems produced comparable total (58.87±5.4 t ha^{-1}), and marketable (48.19 ± 5.1 t ha^{-1}) yields. AGROMAN fruits were larger in comparison to the other systems. AGROCOM system led to lower firmness, acidity and carotenoids than SUBST system and also showed the lowest dry matter.

At harvest, SUBST tomatoes at the breaker stage showed higher vitamin C than AGROCOM, while this difference was not statistically significant after ripening. Pink tomatoes ripened on plant showed higher soluble solids content than pink tomatoes ripened in storage; moreover for AGROCOM and SUBST the carotenoids content was higher when ripened on the plant. In conclusion, in complex systems (AGROMAN and AGROCOM), it was possible to synchronize the mineralization rates of organic amendments and green manure with the needs of the plants, and to obtain similar tomatoes yields and quality of simpler and less sustainable systems (SUBST).

In the case of lamb's lettuce ('d'Olanda'), storage was done in modified atmosphere packaging (MAP) and in air using macro-perforated bags. The bags were stored in a cold room at 4 °C for 11 days. Daily for each bag, oxygen and carbon dioxide partial pressure were monitored. At 0, 4, 7 and 11 days after harvest, weight loss, color (L*, a*, b*, Chroma and Hue°), firmness and chemical parameters (pH, total soluble solids, titratable acidity, ascorbic and dehydro ascorbic acids, antioxidant activity and phenols) were analyzed. Finally, appearance and odor scores were subjectively evaluated after opening the bags.

At harvest, an accumulation of phenols and dehydroascorbic acid in the system with the lowest initial supply of organic amendment (namely SB) was observed. A moderate stress for nutrient starvation has been suggested as possible explanation for this effect. In fact, the AM and AC systems preserved the highest rate of ascorbate pool in reduced form as a result of the fertility management strategy with a long term perspective, based on organic matter rich amendment. Although differences were minimal, AM and AC showed less quality changes over time than SB, whereas regarding the effect of packaging AIR conditions succeeded to maintain the initial quality attributes for a longer period in comparison to the modified atmosphere package that, at 11 day regardless the production system, developed off-odors below the threshold of acceptability. Mainly the initial differences among the production systems were lost or attenuated during storage.

Finally, the potentiality of using f NIR spectroscopy to discriminate among production systems and to predict strawberry internal composition was investigated. Spectral information obtained with FT-NIR spectrometer scanning intact strawberries ‘Festival’, showed excellent potential for the prediction of TSS, TA and pH attributes fruits produced under different fertility management. Phenols could be predicted with lower accuracy, while NIR was less promising for the prediction of antioxidant activity and Vitamin C, reliable as also reported in literature. Moreover a PLS-DA analysis on spectral data allowed to correctly classify the fruits based on the production system.

Further interesting developments of this results may be in fact be aimed to the study of the applicability of this method to identify the authenticity of the production system of strawberries, by increasing the variability of the production factors (cultivar, location, growers, etc) into the model. Moreover this approach can be implemented as a fast, clean methodology that allows growers to improve strawberries quality in the whole production chain, supporting the individuation of the best harvesting time and the selection of clusters of different initial quality.

PART ONE: *GENERAL*

Management of soil fertility and postharvest quality and traceability of organic horticultural products

1. Introduction and general objectives

1.1 Fresh fruit and vegetables: consumers need and market perspectives

International trade in fresh fruit and vegetables amounted to some 250 billion euro per year. The sector is leaded by the European Union (EU) that is both the foremost destination and the primary source of supply worldwide (Malorgio and Felice, 2014). Fruit and vegetables are characterised by strong seasonality and great perishability, therefore research and innovation in postharvest management and technology have always been at the basis of development and advances in this sector. From the production site to the consumption place, the challenge is to maintain the initial quality of the products working against the clock of the biological deterioration (Kader and Lamikanra, 2002).

The major distributors as well as the processors of the fresh-cut produce have implemented careful handling procedures and efficient logistic during all the steps of the supply-chain; they have invested in better storage facilities, appropriate cooling systems and innovative packaging technologies adapting the best available solutions to the specific characteristics of each product. Over the years, the quality of fresh fruit and vegetables on the market has been enhanced and the shelf-life has been extended (Lamikanra, 2002). Meanwhile, food scandals, genetic modifications, animal abuses, label falsifications have spread out food scares among people modifying the food choice criteria. Initially, fresh fruit and vegetables were purchased according the price and their visual appearance and flavour. Nowadays, consumer is looking for healthy food and alternatives diets purchasing produce based on the expected nutritional proprieties, since these are barely indicated in the label (not present for spare products), also because of the strict regulation regarding nutritional and healthy claims on the produce labels (Reg. EC 1924/2006, FDA, 21CFR), that should be based on scientific evidences. Moreover, present certification and traceability procedures and audits shall be supported by food authenticity detection techniques (Georgieva et al., 2013). The introduction of effective analytical techniques for identifying the origin and measuring the quality of products remains something of a challenge for the food industry, it is due to lack of non-destructive methods that are sufficiently accurate not only to identify potential quality differences, but also to authenticate the origin of a given fruit of vegetable in terms of place and production method. Although many manufacturers of on-line grading lines are implementing NIR systems to measure various quality attributes (Nicolai et al., 2007) costumer demands are far to be fully satisfied.

Day by day, the awareness about the importance of food quality in the developed regions of the world is growing, but in a wide picture, the actual challenge is to ensure adequate diets nutrition for all, especially in developing Countries. Surely, food security is based on food availability and access but also on food safety and nutritional quality. The 1st of April 2016, the United Nations agreed a resolution proclaiming the decade of action on nutrition from 2016 to 2025 aiming to eradicate malnutrition worldwide, and ensure universal access to healthier and more sustainable diets for all people, whoever they are and wherever they live (UN, 2016).

The nutritional quality of a fresh produce on the market depends on the initial quality to a large extent; it is a matter of fact that, after harvest the quality can only decrease. For this reason, to delay as much as possible quality losses, most of retailers are improving their standards at the reception as also fresh-cut processors are selecting healthier row materials. Growers are therefore called to supply extra and first quality fruits and vegetables taking care of all the factors that may improve production quality at farm level (Francis, 2012). To enhance the nutritional quality of fresh and fresh-cut products more attention should be directed towards obtaining fresh produce with superior quality in terms of vitamins, antioxidant activity and, in general, secondary metabolites.

Genotype and rootstock, pedoclimatic factors, cultural practices, maturity and ripening at harvest, greatly affect the quality at harvest of fresh fruits and vegetables. Each of these factors, in single or combination effect, have been widely studied on several crops, but is hard to link the all-inclusive cause and effect relationship of a well-defined production system to appealed quality characters (Kader, 2000). Furthermore, whether the postharvest evolution of the initial quality attributes might be modulated, by a specific set of pre-harvest factors, and in this latter case up to which extent, is far to be completely understood.

1.2 Organic fruits and vegetables

A much active debate in the scientific literature is taking place concerning the nutritional quality of food organically produced. Proponents of the organic production claim the superior quality of fresh fruits and vegetable produced in organic farming systems. Consumer seems to trust this hypothesis also because following food scandals, the general concern for the environment increased as also public debates on sustainability issues.

Organic agriculture is practiced in 172 countries, and 43.7 million hectares (1% share of total agricultural land) are managed organically by approximately 2.3 millions of farmers. The organic market size of organic food and drinks reached 70 billion of euro in 2014. Organically grown fruits and vegetables, both whole and fresh-cut represent a growing segment in this sector (FIBL and IFOAM, 2016).

In EU, Reg (EC) No 834/2007 defines organic farming principles (art.5) and rules (art.12). Among the most important principles: the maintenance and enhancement of soil fertility and biodiversity feeding the plants primarily through the soil ecosystem; the minimisation of the use of non-renewable sources and off-farm inputs; the recycling of wastes and by-product as input; the maintenance of plant health by preventative measures, such as the choice of appropriate species and varieties resistant to pests and diseases; the appropriate crop rotations; the application of mechanical and physical methods and the protection of natural enemies of pests. The local or regional ecological balance shall also be considered when taking production decisions (EC 834/07). The following rules shall apply to organic plant production: tillage and cultivation practices shall prevent soil compaction and erosion and they shall maintain or increase soil organic matter, stability, biodiversity, soil fertility and biological activity, by implementing multiannual crop rotation including legumes and other green manure crops, and by the application of livestock manure or organic material, both preferably composted, from organic production. All plant production techniques used shall prevent or minimise any contribution to the contamination of the environment (EC 834/07). Moreover, in the Reg (EC) No 889/2008 are listed the technical tools and products that are allowed in plant nutrition (Annex I) and protection (Annex II).

In more detail, to comply with the aforementioned principles and rules, organic farmers shall set up an effective fertility management strategies focusing at three keystones: crop rotation, organic matter and organic fertilizers. The key objectives of crop rotation design and plan are breaking disease and pest cycles and increase crop diversity including legumes (soil fertility building crops) and cover crops(Tittarelli et al., 2016). Different crops with different root systems will explore different soil niches, ameliorating structure and nutrients availability. To this aim the introduction of agroecological services providing crops (ASC) in the rotation is a possible asset. ASC crops are no cash crop species, that are cultivated to provide or enhance environmental services of the agro-ecosystem (Thorup-Kristensen *et al.* 2003). They maintain or increase soil organic matter content and nutrient availability for further crops. The ASC belonging to *Fabaceae* family supply nitrogen into the cropping system (Ciaccia et al., 2015); when belonging to *Poaceae* are strong biomasses producers and are breaking the diseases and pests cycles whether the cover crops are not alternate hosts (Canali et al., 2015); finallu the ASC belonging to the *Brassicaceae* family produce sulphur compounds preventing soil borne diseases. Usually, nutrients input for organic farming are grouped into organic amendments, base and dress fertilizers (Möller & Schultheiß, 2015). Organic amendment (animal manure, compost and digestate) refers to solid fertilizers applied in huge quantity before soil bed preparation. On average, they have low nutrient concentrations on fresh weight basis but they contain relevant amount of organic matter at various maturity level. Base fertilizers are much richer in nutrient than the amendments, they are commercial organic fertilizers which may include also organic amendment and mineral fertilizers (not chemically synthesised, such as natural rock) in their compositions. Complementary fertilizers are commercial fertilizer usually in liquid form that easily supply soluble nutrients. The complementary fertilizer may be applied during plants growth to support crop production. All this inputs are listed in the Annex I of Reg EC, 889/2008 that reports an heterogeneous collection of different raw materials, nutrient compositions, and, accordingly, mineralization rates. Due to their organic origin, they are multi-nutrients supply which easily do not comply with the nutrient demand of the plants, creating unbalanced fertilization for one of more elements (Voogt, 2011). Also, for this reason the synchronization of mineralization rate and plant demand of nutrient is a goal hard to reach and to keep during the whole cultivation period (Ceglie and Abdelrahman, 2014). It is evident that principles concerning off-farm inputs, nutrients recycling, environmental impact and non-renewable resources may not be objectively standardized in the Regulation and simply verified from the inspection bodies. Consequently, to the full compliance of the rules, that permits the certification of organic products, correspond a certain degree of observance of the organic production principles. In other words, organic method is based on a series of very diverse approaches: sequence of crop rotation, sources and quality of inputs, timing and doses of water and fertilizers, weed management and cover crop uses, pathogen and pest control. Frequently, organic farmers implement a substitution approach (namely "conventionalized" system, according to Goldenberg, 2011) in which the nutritional needs or lacks are treated "substituting" off-farm synthetic inputs with off-farm organic inputs (De Wit and Verhoog, 2007), that shall be reported in the Annex I of Regulation. In the organic substitution systems, the fertility management relies on short term perspective by applying external input to return the nutrient removed by crop uptake. An alternative soil fertility management approach relies on recycling on-farm input and enrich soil organic matter in a long term perspective. In this way the fertility management aims to fulfil the organic farming principles much more than the "conventionalized" system which, anyway, still comply with the organic rules (Darnhofer et al., 2010). This approach implements crop rotation to increase crop diversity, including ASC, and provides organic matter based nutrient to the soil (by amendment, cover crop and crop residues incorporation) to feed the crop by organic matter mineralization. Those strategies represent two dichotomous visions of the organic production method; each of them might be chosen by famer, according to technical, financial and marketing opportunities. At farmers level, both this approach may be implemented at a certain extent resulting in a wide heterogeneity of the organic production systems that are actually implemented. Similarly, this is also true in conventional production system, where it is possible to have high input-intensity or moderate input intensity farming systems. It results a complex picture in which organic and conventional farming can't be defined as two isolated and different clusters of agronomic practices. For this reason it is quite tricky to compare quality of organic certified products with the conventional counterpart because the variability of the pre-harvest conditions can't be easily standardized to study the eventual differences between conventionally and organically grown products.

Focusing the attention of the fertility issues, the main purpose of conventional system is the nutrition of the crop to sustain and improve the yield and to ensure availability of mineral nutrient in time and amount, taking into account the macro-micro nutrients relative ratio required by the specific commodity (as the example of the nutrient solution supplied for soilless cultivation). Indeed, the primary target for organic farming systems is to maintain and improve the soil fertility management, and once obtained, the secondary target is the plant nutrition that will be mainly supported by the soil mineralization process itself (in fact, soilless production is not allowed in organic farming, according to EU regulation). However, high nutrient uptake, especially in fruit and vegetables production, needs high inputs application. Off-farm input shall be applied as described in the Annex I of 889/2008. EU regulation for organic productions does not limit the quantities of fertilisers, however, the maximum supplied level of fertilizers from animal origin per year is 170 kg N/ha (according the nitrate directive; EEC, 91/676/EEC). This limitation may lead growers to use high quantities of composts or other organic fertilisers, of vegetal origin, to meet the requirements of the cropping plan.

Fertilization influences not only crop performance and crop yields, but also crop quality (taste, firmness, internal quality, storage performance). Limited data are available about the influence of the growing conditions and the fertility management strategy on the quality attributes of organic crops. Recent studies, suggesting that organic farming management may create moderate stress conditions for the plants, that in response to this accumulate sugars and secondary metabolites such as vitamin C and phenolic compounds improving the nutritional quality of organic production (Oliveira et al., 2013). As a consequence, different type and amount of secondary metabolites at harvest may affect the antioxidant activity during storage period. This might determine diverse postharvest performances in respect to the different fertility management strategies implemented to produce organic or conventional fruits and vegetables. Despite the relevance of this research hypothesis, few comparison studies are focused on postharvest investigations under this perspective.

1.3 Objectives

Standing to these considerations, the general objectives of this research were:

- To verify that the implementation of agro-ecological approaches to soil fertility management does not affect organic yield and quality potentials in respect to the substitution approach, tested on strawberry, as model crop;
- To study the effect of soil fertility management strategies on quality at harvest and during storage of tomatoes, strawberries and corn salad organically produced ;
- To compare the quality attributes at harvest and during storage of strawberry fruits, as model crop, organically and conventionally produced in similar pedoclimatic conditions;

- To investigate the potentiality of NIR methodology to classify strawberry fruits grown under different production system and to predict the internal composition with a non-destructive approach.

Strawberry was chosen as crop model for many experiments, due to its perishability during postharvest operations which may be critical for organically grown fruits. Moreover for this crop it was possible to find a control of conventional fruits of the same variety, grown locally in the same pedoclimatic conditions.

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2. Effect of Organic Production Systems on Quality and Postharvest Performance of Horticultural Produce[1]

Abstract

Organic standards include a well-defined set of practices and a list of technical tools that are permitted by the Regulations. Organic products are mainly purchased for their safety and absence of pesticide residues. Furthermore, a diet based on organic products claims to provide health benefits due to the high nutritional values compounds that are more concentrated in the organic products compared to conventional ones. As the scientific basis of the differences between organically and conventionally produced fruit and vegetables are under debate some of the published work, together with some recent unpublished results, will be covered in the present review. In addition, the effect of different approaches to the organic horticultural production will be described. Many publications confirmed lower nitrate content, especially in leafy vegetables, and higher antioxidant compounds in organically grown fruits in comparison to the conventional ones. A recent study reported organic kiwifruits as higher in ascorbic acid and total phenol contents than conventional fruits. These differences were maintained throughout storage duration. Similarly, in organic grapes, antioxidant-related compounds were significantly higher than the conventionally grown grapes. Analogue results were obtained for organic strawberries grown in protected conditions. However,

[1] *Ceglie, F. G., Amodio, M. L., & Colelli, G. (2016). Effect of Organic Production Systems on Quality and Postharvest Performance of Horticultural Produce. Horticulturae, 2(2), 4.*

conventional products usually result in higher moisture content and this should be taken into account to confirm the differences also in terms of dry matter basis. Possible explanation for the effects of organic farming practices on nutritional quality and postharvest performance of fresh produce are the following: (i) organic amendments provide high input of exogenous organic matter and of nutrients for a long period; on the contrary mineral fertilizers, allowed only in conventional farming systems, are highly concentrated of nutrients that are directly available for the root uptake; (ii) the use of chemical pesticide (only possible in conventional agriculture) slows down the defences against pathogens molecular pathways of the plants, with the consequence of favouring the primary metabolism; (iii) cultural practices may result in diverse plant composition and nutritional quality, which in turn influence storage performance of the products as these differences, both in fertility and pest management, affect C allocation to secondary plant metabolites (such as ascorbic acid and phenolic compounds).

2.1 Introduction

Organic standards include a well-defined set of practices and a list of technical tools that are permitted by the Regulations (i.e. Reg n.889/08 in UE and the National Organic Program in US). A diet based on organic products claims to provide health benefits due to: high nutritional value compounds that are more concentrated in the organic products compared to conventional ones; absence of pesticide residues (Oaetes et al., 2014). The present challenge of feeding the world requires new strategies to ensure food security which is surely based on food availability and access but also on food safety and nutritional quality. Organic production system has the challenge to keep together both the sustainability of the production, allowing conservation of natural resources for present and future generations, and the high quality and shelf life performances of the organic product (Rembialkowska, 2007). Despite the importance of the issue, few comparison studies are focused on postharvest investigations and the scientific basis of the differences between organically and conventionally managed produce is under debate (Smith et al., 2012). Some of the published works, together with some recent unpublished results, will be covered in the present review. In particular, the comparison studies that are conducted under similar environmental conditions in terms of climate, soil characteristics, availability of nutrients, have been considered for the effect of diverse pre-harvest practices on postharvest performances.

2.2 Diverse plant response to different systems of production

The overall quality characteristics of the plant parts which are used as food is the result of the interaction among genotype, environmental conditions cultural practices and postharvest process and technology. Any environmental variable that upset the plant from the ideal growing conditions generate a stress which leads to either activate or inhibit specific plant responses. Any stress pressure may lead back to oxidative stress that produces reactive oxygen species (ROS) in the chloroplasts (free radicals like superoxide and molecular forms like singlet oxygen) and in the mitochondria (mainly the superoxide). Ascorbic acid, glutathione, phenolic compounds and alkaloids, among the others antioxidats, are the non-enzymatic defense system the plants uses to cope with this dangerous situation (Gill and Tuteja, 2010).

In general, an organic production system is a stressful system largely due to insufficient supplies of mineral nitrogen throughout the crop cycle, fostering phenolic and ROS which are natural defense substances. For organic plants, the content of secondary metabolites, that also include antioxidants, have been positively correlated with this natural defense substances (Winter et al., 2006). However antioxidant biosynthesis requires a plant resource relocation from the primary metabolism to the secondary metabolites production. In agricultural crops the plant structure is the primary production determining the yield. As a consequence, cultural practices, both organic and conventional, may result in diverse plant composition and nutritional quality at harvest due to different biotic and/or abiotic stress conditions, which in turn influence the concentration of secondary metabolites in the final produce.

2.3 Quality and postharvest performances of organic and conventional fruit and vegetables

In the scientific literature different publications are claiming for opposite results. Woëse *et al.* (Woese et al., 1997) and Smith Spangler *et al*. (Smith et al., 2012) estimated large but not statistically significant differences between organic and conventional managed crops, which leads to conclude that this two production systems had similar qualitative characteristics. However, in the last decade, further papers have contributed to enlarge the dataset of this comparison. Lairon (Lairon, 2010) confirmed the absence of pesticide residues in organic food (97% of samples were under detectable level of pesticide) and found out the lower content of nitrogen in organic vegetables in comparison to conventional ones. In this respect, Baranski et al. (Baranski et al., 2014) were able to conclude that concentration of total nitrogen was 10% lower in organic compared to conventional crops (nitrate 30% and nitrite 87% lower) (Caruso et al., 20111). The low percentage of nitrogen forms seems to be correlated with high concentration of secondary metabolites (such as phenols and vitamins, which are no nitrogen-containing compounds). Several authors have confirmed elevated levels of secondary metabolites in organic carrots (Sikora et al., 2009), sweet peppers (del Amor et al., 2008) and tomatoes (Pieper and Barret, 2009). Other studies have concluded with opposite results in case of tomatoes (Rossi et al., 2008) and carrots (Krejcova et al., 2016). The plant secondary metabolites are taking place for their role in enhancing human health (Lundegardh and Martensson, 2003), as a consequence, much attention has been dedicated to this family of compounds in the recent comparison studies. Luthria (Luthria et al., 2010) found similar phenolic acids content in eggplants cultivated with organic or conventional techniques. Chassy (Chassy *et al*, 2006). reported higher values of antioxidant activity in organic tomato and bell peppers while D'Evoli *et al.* (d'Evoli et al., 2010) found that total polyphenol content was higher in conventional plums, but ascorbic acid, α-tocoferol and β-carotene were higher in organic samples. Furthermore, the majority of studies comparing the polyphenol content in plant products from different farming systems indicated significant higher concentrations of these substances in organic fruits and vegetables. Lairon (Lairon, 2010) highlighted the higher level of antioxidants, minerals, and dry matter in organically grown products. However, conventional products usually result in higher water content and this should be taken into account to confirm the differences also in terms of dry matter. Bourn and Prescott (in 2002) observed higher dry matter content in organically grown carrot and spinaches in comparison with the conventional ones. Ceglie *et al.* (in 2014) found higher dry matter content in organic strawberries that are probably due to the higher content of water absorbed by the conventional products, as also confirmed by Conti *et al.* (2014). Dry matter at harvest has been proposed as a promising predictor of the post-harvest soluble solids in apples and the initial difference in dry matter may affect the relative amount of mass that could be lost during storage (McGlone et al., 2003). Woëse *et al.* (in 1997) in their review reported no clear trend in the level of total sugar content in organic vegetables compared with conventional ones.

Different type and amount of secondary metabolites at harvest may affect the antioxidant behavior during the storage period and determine diverse postharvest performance of the products.

Despite the relevance of the issue, few comparison studies are focused on postharvest investigations. Differences during conservation in terms of: incidence of physiological disorders, soluble solids, firmness and mineral content have been reported in few studies (DeEll and Prange, 1993; Weibel et al., 2002). After ethylene treatment, organic banana showed faster peel color changes, lower gravimetric pulp/peel ratio and impedance, in comparison with conventionally grown banana (Nyanjage et al., 2001). Organoleptic characteristics, and resistance to deterioration resulted higher for organic strawberry in comparison to conventional fruit during simulated marketing conditions (Cayuela et al., 1997). Hasey *et al.* (in 1997) reported higher soluble solids and firmness in organically grown than in conventional kiwifruits. On the contrary, Benge *et al.* (in 2000) reported that conventional kiwi fruits showed higher soluble solid content and similar softening behavior and decay of the organic ones, analyzed at the same firmness stage. The levels of calcium in kiwifruits were negatively correlated with incidence of soft patches. Also high levels of Ca as well as of NO_3^-, Mg, Fe and Zn was observed at harvest and during 25 days of storage at 10 °C in conventional 'Meyer' lemons (*Citrus meyeri* Tan.) in comparison with fruit from organic orchard (Uckoo et al., 2015).

Further contributes to investigate the effect of organic and conventional farming systems on postharvest performances are hereby presented in two research cases on grapes (unpublished) and kiwi fruit (Amodio et al., 2007).

2.4 Postharvest performance of organically and conventionally grown kiwifruits.

Amodio *et al.* (in 2007) compared postharvest performance of organic and conventional 'Hayward' kiwifruits harvested at the same maturity stage. Fruit shape and peel characteristics before storage, maturity indices (CO_2 and C_2H_4 production, firmness, color, soluble solids content and acidity) and compounds associated with flavor and nutritional quality (minerals, sugars and organic acids, ascorbic acid, total phenols, and antioxidant activity) were determined at 0, 35, 72, 90 and 120 days of storage at 0°C, and after 1 week of shelf-life simulation at 20°C.

At harvest, organically and conventionally grown kiwifruits had similar soluble solids content; conventional kiwifruits had a higher firmness and L* value, and a lower hue angle and chromaticity, resulting in a lighter green color when compared with the organic kiwifruits. During storage, soluble solids content increased more in the conventional than in organic kiwi fruits. Concerning nutritional compounds, ascorbic acid and total phenol were higher in organically grown kiwi fruits than in conventional ones resulting in a higher antioxidant activity, these differences were maintained throughout storage period. The two production systems resulted in different morphological attributes since organic kiwifruits exhibited a larger total and columella area, smaller flesh area, more spherical shape, and thicker skin compared to conventional kiwi fruits. Finally, all the main mineral constituents were more concentrated in organic kiwi fruits.

2.5 Postharvest quality of organic and conventional white table grapes.

The effects of organic and conventional table grapes grown in two locations of Apulia Region were evaluated at harvest and during 14 days of cold storage at 0 °C (Amodio et al., unpublished). Respiration rate, firmness, color, soluble solids content, acidity, organic acids, ascorbic acid, total phenols, antioxidant activity flavonols, peel characteristics were determined at harvest and during storage.

Only in one location, phenolic compounds were significantly higher in the organic table grape (505.1 ± 52.4 mg gallic acid/100 g) than in the conventional ones (369.8 ± 57.8 mg gallic acid/100 g). Similar behavior was reported for the antioxidant activity that during harvesting span was higher in organic grapes

(1210.7±134.3 mg Trolox/100 g) than in conventional ones (763.4± 97.6 mg Trolox/100 g). This difference was observed until the 6th day of storage. Vitamin C in organic and conventional table grapes presented similar contents, without significant differences both at harvest and throughout storage. Storability was greatly affected by the agricultural system resulting in a shorter shelf-life of organic grapes which scored the highest values of firmness and appearance scores.

2.6 Organic vs. conventional products: comparative analysis in a 'cul de sac'

Organic and conventional production systems are made of a series of very diverse approaches: sequence of crop rotation, sources and quality of inputs, timing and doses of water and fertilizers, weed management and cover crop uses, pathogen and pest control. Organic and conventional farming can't be defined as two isolated clusters of agronomic practices. On one hand, it is possible to have high input-intensity conventional farming or moderate input intensity conventional farming systems. An example is when the use of chemical inputs is restricted by private standards (for commercial reasons) such as the 'zero pesticide residues' labels. On the other hand, organic farming should rely on a more complex agro-ecosystem management that includes leguminous crops and organic matter-based amendments, but it is possible that implements a simple input substitution based on the replace of chemical fertilizers and pesticides with organic-allowed input. In scientific literature this process in known as "conventionalization" of organic farming (Goldenberger, 2011) which may be summarized in the development of organic farming practices that might not be sustainable but that are not excluded by the organic standards (Padel et al., 2007). In particular, the case of organic greenhouse production is a clear example of this. The high intensive production level recorded in greenhouse systems requires high availability of nutrients to sustain the crop growth; as a consequence organic growers simplify the crop rotation, exclude cover crops and use easily soluble organic fertilizers. All these issues should be taken into account also in the case of a study that aims to compare the quality and post-harvest performances of organic vs. conventional farming systems. The comparative analysis have to face the heterogeneity of the circumstances: observed difference and/or similarity would not necessarily be related to certification systems of the products because organic certified products might have been produced by an organic conventionalized system and vice-versa. If organic-conventional system comparisons implemented the same agronomical practices and only varied in the type of nutrient or pesticide input, this would not be an organic vs. conventional system comparison but a comparison of organic vs. conventional inputs (Seufert et el., 2012).

In this respect, there is room to begin comparative analysis among different approaches to organic method of production in order to appreciate, within the much heterogeneous cluster of organic practices, the impact on the quality of an organic conventionalized system vs. other organic systems which are based on agro-ecological practices. Such comparison studies should be considered as investigation of the effects of pre-harvest practices on the post-harvest quality and products shelf life. In this framework, two research cases are shortly reported as preliminary results on this promising research line.

2.7 Effects of different systems of organic production on quality and post-harvest of tomato

Tomato, a climacteric fruit, represents a significant source of folate, vitamin C, polyphenols and other antioxidants (Charanjeet et al., 2004). A conventionalized organic production system based on organic commercial fertilizers was compared with two organic production systems based on agro-ecological practices represented by (i) animal manure amendment and dead mulch of cover crops; or (ii) on-farm compost amendment green manuring of cover crops. For the three production systems, tomato fruits respiration rate, morphological, physical, sensorial characteristics, and nutritional compound content were monitored. It results that dehydroascorbic acid was significantly higher in the conventionalized system for tomato fruits harvested at breaker and pink stage. However this difference disappeared after ten days of storage at 15 °C. Organic agro-ecological systems were able to obtain a similar tomato yield and fruit quality than organic conventionalised system which used off-farm inputs only. This was confirmed both at harvest and during storage. Further information and results of this experiment are in literature (Ceglie et al., 2015). It is worth noting that when the environmental condition are similar in terms of climate, soil characteristics, availability of water and soluble nutrients for the roots, any difference in the practices (even relevant such as cover crop mixtures vs. bare soil, organic dead mulch vs. plastic mulch, manure vs. commercial organic fertilizers) did not affect the crop quality and postharvest performances.

2. 8 Effects of different systems of organic production on quality and post-harvest

of strawberry

Ceglie *et al.* (in 2014) compared quality characteristics and postharvest performance of organically and conventionally produced strawberry fruit. The production system affected the quality parameters of 'Festival' strawberry cultivated in unheated tunnel. Organically grown strawberry resulted in higher Vitamin C, total soluble solids, sugars (sucrose and fructose) content and aroma values than conventionally grown strawberry which, on the other hand, showed a better firmness and appearance. Similar results were obtained for different varieties in the same geographic area (South of Italy) as reported by (Conti et al., 2014). The experiment compared three organic farming systems (two "agro-ecological" and one "conventionalized") and a conventional one. Conventionally grown strawberry fruits resulted higher in diameter, Chroma values and texture compared to the organically produced fruits regardless the organic system considered. Vitamin C, malic acid, tartaric acid, fructose and glucose contents were higher in the organic strawberry compared to conventional fruits. The latter received higher scores in terms of appearance, color and acidity, while the organically grown fruits showed higher sweetness and aroma and lower off-flavor scores. During the whole period of cold storage titratable acidity, citric acid and total phenols values presented a similar decreasing trend in all the growing systems under investigation. Moreover, the two organic agro-ecological systems presented a different behavior in comparison to both the "conventionalized" organic and the conventional system of production. At harvest, ascorbic acid and sucrose concentrations were higher in both the organic agro-ecological than in the other two systems. Furthermore, during the whole storage period tartaric acid and total phenols resulted higher in the organic agro-ecological systems than in both the conventional and the "conventionalized" organic systems. Under this perspective, a conventionalized organic system may result somewhat at the halfway between conventional farming and organic-agroecological systems both in terms of quality and in terms of environmental sustainability. In this respect, also Reganold *et al.* (Reganold et al., 2010) linked the high quality of organic strawberry fruits with the high capability and stress resilience of organically managed soil. Further studies on other commodities and with a longer term assessment perspective are necessary to individuate sets of cultural practices applicable under the organic regulation which may enhance the quality of the organic produce.

2.9 Conclusions

This review represents a small contribution to draw the picture of the quality of horticulture produce in relationship to the system of production. Organic production system has the objective to include a rational use of natural resources with a high quality level and shelf life performances. In many cases, also conventional farming systems have been reported to achieve such high results. Nowadays, organic agriculture is increasing in terms of invested surface and number of operators. This originated a wide set of solutions which, although valid with respect to the organic certification standards, still need scientific assessments concerning the claimed sustainability and high quality performances. A more in deep analysis may relate the organic vs. conventional comparison to the more general issue of pre-harvest effects on postharvest performance of crops. In this respect, the balance between primary and secondary metabolic pathways seems to be an effective key to re-discuss the complex interaction of genotype, environment and agricultural practices which lead to different quality and postharvest performance of fresh fruits and vegetables. The need to improve the quality of food available in the world should orient agricultural practices to increase the nutritional composition of fresh fruits and vegetables and to enhance the shelf life projections.

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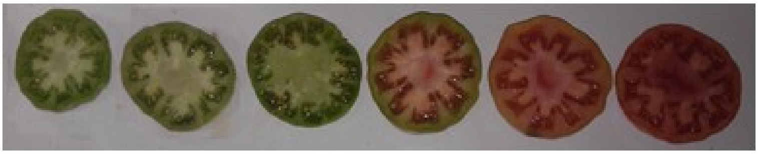
PART TWO: *EXPERIMENTAL*



Management of soil fertility and postharvest quality and traceability of organic horticultural products

3. Organic strawberry in Mediterranean greenhouse: effect of different systems of production on soil fertility and fruit quality[2]

Abstract

In Europe, the lack of specific rules regulating organic vegetable production in protected conditions has led to the implementation of extremely diversified systems of production, at different level of intensification. In our research, we compared three strawberry organic systems of production based on the following main criteria of soil fertility management: input substitution (SB), a simplified system of organic production that mimic the conventional agricultural practices and two systems characterized by a more complex soil fertility management, based on the introduction, in the rotation, of agroecological service crops (ASCs) and compost (AC) and ASCs and cattle manure (AM). Strawberry yields, in the compared treatments, were not significantly different in both years of our research, while, as a whole, the yield in 2013 (30.3 Mg ha^{-1}) was significantly higher than in 2014 (28.9 Mg ha^{-1}). Crop nitrogen needs during the entire cycle of production were satisfied according to the same pattern by SB, AC and AM, while green manuring and organic amendments in AM and AC determined a higher soil organic N content compared to SB. As far as the production quality is concerned, AM and AC allowed to obtain strawberry fruits similar to SB, but with better characteristics in terms of

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color and phenolic content. AM and AC did not differentiate statistically in the two year period of our research.

3. 1 Introduction

In the context of global markets, where the demand for off season vegetable production is increasing, greenhouse production has the potentiality to become a niche market for organic products (Tüzel et al., 2013). As known, in the EU, organic production is ruled by specific regulation, but neither in regulation (EEC) n. 2092/91 (EEC, 1991) and its replacements Council Regulation (EC) n. 834/07 (EC, 2007) nor in Commission Regulation (EC) n. 889/08 (EC, 2008), direct reference is made to greenhouse production. Lack of legislation has determined the implementation of national rules derived from the interpretation of the general regulation on organic farming (van Der Lans et al., 2011). Due to the high level of intensification of greenhouse production, this lack of specific normative has determined the widespread use of "conventionalized" systems of production, based more on a simplified approach of input-substitution rather than the respect of the basic principle of organic farming (Guthman, 2004; Darhnoffer et al., 2010). If, from one side, a "conventionalized" approach to organic production has allowed in recent years a significant growth of yield (Voogt et al., 2011), from the other side, has increased the risk of implementing a simplified system of organic production that mimic the conventional agricultural practices. Thus, organic farms could develop practices not excluded by the organic norms but not sustainable in the long term (Padel et al., 2007). Indeed, under these stressing and exceptional conditions, it has been proven that high input agricultural systems (either organic and conventional) are not resilient (Foley et al., 2011).

One of the more controversial issues of debate, in organic greenhouse vegetable production, is soil fertility management. As a general consideration, soil fertility management in organic production is much more complex than in conventional systems. It should be based on agro-ecological strategies requiring deep knowledge of the pedoclimatic situation and of nutrient flow through the agro-ecosystem. Soil fertility should be influenced and regulated by crop rotation, green manure, animal manure and/or compost application integrated with low input organic fertilizer. The introduction of cover crops in the rotation may also contribute to maintain and promote soil fertility (e.g. increase of soil nutrient availability; reduction of soil erosion) and provide other so-called agro-ecological services (like pest and weed control). For this reason, the most recent scientific literature defined them as Agro-ecological Service Crops (ASCs) (Canali et al., 2015).

Another important aspect of soil fertility management is the estimation of mineralization rates of organic biomass and their synchronization with plant needs. In this context, the use of simplified nutrient budgets may allow the evaluation of short and long term soil fertility management strategies (Tittarelli et al., 2014; Montemurro et al., 2015).

Moreover, there is a need of defining the agronomic practices able to guarantee both high quality and nutritional content of the production with a low amount of external inputs (Rembiałkowska, 2007).

In this productive context, a long term experiment has been carried out with the main aim of individuating a Mediterranean organic greenhouse production system which can be sustainable in terms of yield and quality productions. In order to reach this objective, a conventionalized system was compared with two alternative (agro-ecological) systems based on more complex fertilization patterns, characterized by combination of green manure, compost, cattle manure and commercial organic fertilizers.

Particularly, the following specific goals were pursued: (i) to verify the hypothesis that the implementation of agro-ecological approaches to soil fertility management does not affect organic strawberry yield and quality potentials in greenhouse; (ii) to verify the hypothesis that agro-ecological systems are able to synchronize soil mineral nitrogen availability with the plant needs along the cash crop cycle; (iii) to assess short and long term fertility of the compared systems (iv) to assess fruit quality differences.

3.2 Materials and Methods

Experimental site

The research was carried out at the experimental farm of the Mediterranean Agronomic Institute of Bari (MAIB), in Valenzano (Apulia region- Southern Italy). The location was about 72 meter above sea level, (41°08' N latitude and 16°51'E longitude). The experimental greenhouse (300m^2- 7.5m x 40.0m)was an un-heated tunnel (EUROPROGRESS s.r.l. –Italy) with galvanized steel frames covered by ethylene vinyl acetate (EVA) sheets. It was divided into two fields (field I and field

II), cultivated with tomato and strawberry in rotation, in 2012-2013 and in 2013-2014. The present research was based on two cycles of strawberry cultivation (September 2012– May 2013 and September 2013 – May 2014 in field II and I, respectively).

Experimental design

The organic farming systems under comparison were: i) SUBSTITUTION (SB), a widely adopted organic production system (very diffused especially in greenhouse vegetable production), which mimics conventional agriculture by substituting agrochemicals with allowed organic products; ii) AGROMAN (AM), characterized by the use of a mixture of ASCs (MIX 1) and mature organic manure as soil amendment, and iii) AGROCOM (AC), which utilizes a different mixture of ASCs (MIX 2) and, as soil amendment, on-farm made compost. The composition of the two ASCs mixtures is shown in Table 1. The experimental layout was a completely randomized block (CRB) design with three replication (9 plots of 3.0 m x 4.0 m each). The choice of mixtures, instead of the cultivation of single ASC species, was done in order to better guarantee the provision of the ecological services in the long run. ASC mixtures were broadly sown the 6th and the 15th of June in 2012 and 2013, respectively, and chopped and ploughed into soil as green manure after 50 days of growing in both the years.

Strawberry (*Fragaria* × *ananassa* var Duchesne, ‘Festival’) was transplanted on the 22nd September and on the 4th October in 2012 and 2013, respectively, using certified seedlings from organic nursery (Vivai f.lli Zanzi – Ferrara, Italy).

Density of strawberry crop was 3.25 plants m^{-2}, (0.9 m between lines and 0.3 m within each line). On the first year, harvesting started on the 11th March and ended on the 7th May 2013. Strawberry was harvested at fruit ripening at 171, 179, 187, 196, 200, 205, 207, 211, 218, 224 and 229 days after transplanting (DAT). On the second year, harvesting started on the 22nd March 2014 and ended on the 9th May 2014. Strawberryfruits were harvested at 169, 174, 180, 185, 188, 192, 195, 200, 206, 210, 214 and 217 days after transplanting (DAT). Cumulative production (Yield) per system was then calculated for each experimental year.

Soil preparation

The tunnel greenhouse was installed in May 2012, on a soil organically managed for ten years. Soil was left fallow for two years, before the beginning of this experiment, then ploughed by a rotary tiller.Soil was sampled at time zero to determine physical and chemical characteristics, reported in Table 2. In both the experimental years, the same agronomic practices were applied. In particular, before strawberry transplanting, soil bed was prepared with three windrows for each plot. The water supply system was placed on the top of the windrows for drip irrigation. The whole soil bed was covered by black polyethylene plastic as mulch. On each windrow one line of strawberry was cultivated.

Organic amendment and fertilizers

Total amount of amendments and fertilizers applied to the three systems and their chemical composition are reported in Tables 3 and 4, respectively. A liquid organic fertilizer, based on vegetal proteins, has been used during the plant growth. Compost and cattle manure samples were analysed in triplicate for dry matter, organic matter (OM) and total organic carbon (TOC), total nitrogen (TN), phosphorous (P) and potassium (K_2O). Dry matter was calculated by weight loss overnight in oven at 105°C.OM was determined by ignition of 5 g of samples in a muffle furnace at 550°C and TOC was calculated by dividing OM to the coefficient of 1.92 according to ISPRA methods (2001). TN was analysed according to the Kjeldahl method. After mineralization of 0.5 g of samples, total P was determined by a spectrophotometer (model Megatech SP9) and K_2O was analyzed by flame photometer (Sherwood 410 microwave digester CEM model).

Plant sampling and analysis

Each year, at the end of the ASC cycle, three quadrants (0.25m x0.25 m) per plot were used to sample the fresh aboveground biomasses. Strawberry fruits, at the same stage of maturity at each harvest, were collected from the plot and divided into two subsets. One part was dried and stored for nutrient analysis and the other one was quickly delivered at the post-harvest laboratory of the University of Foggia (Italy) for quality evaluation. Strawberry aboveground residues were sampled at the end of each cropping cycle. The aboveground biomasses and fruits were divided into two parts: one was dried at105°C for the determination of the dry matter content by gravimetric loss, while the other was dried at 60˚C for carbon and nitrogen determinations. On ASC samples, organic matter was determined by ignition in a muffle furnace at 550°C, then organic carbon was calculated according to ISPRA methods (2001).Total nitrogen was analysed by Dumas method using the elemental analyser Turbo Nitrogen (Perkin-Elmer series II 240).

Fruit Quality

The maturity stages of the fruits were visually evaluated according to the protocol of the Agricultural Marketing Service and Vegetable Division (United States, Fresh Products, 1997). Colour was measured on two sides of the fruit, using a colorimeter (CM-2600d , Minolta, Osaka, Japan) in the CIE L*, a*, b* mode, and then, hue angle, Hue°=arctan b^{*2}/a^{*2} and chromaticity, Chroma=SQRT($a^{*2} + b^{*2}$), were calculated. Firmness was determined on ten strawberry fruits for each replicate as percentage of diameter deformation under a force applied of 5N (kg m s^{-2}) between two parallel plates using an Universal Testing Machine (INSTRON 3343 Norwood, MA, US).

Strawberry juice was obtained by squeezing 5 g of fruits for each replicate. Juice drops were used for direct readings of total soluble solids percentage (TSS), by a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan) while 2 g of juice were used for pH and TA measurements using an automatic titrator (TitroMatic CRISON, Spain) with 0.1 mol L^{-1}NaOH solution up to pH 8.1. TA was reported as percentage of citric acid per 100mL.

Total phenol content (TotPh) was determined on 5 g of fruit tissue extract according to the method Singleton and Rossi (1965), slightly modified (Amodio et al., 2014). The content of total phenols was calculated on the basis of gallic acid calibration curve and expressed as mg gallic acid kg^{-1} of fruits fresh weight.

Vitamin C content (VitC) was assessed homogenising 5 g of fruits for 1 min with 5 mL of methanol/water (5:95), plus citric acid (21 g L^{-1}), EDTA (0.5 g L^{-1}), NaF (0.168 g L^{-1}). The homogenate was filtered through cheesecloth and the pH adjusted to 2.2 – 2.4 by addition of 6 mol L^{-1} HCl. The homogenate was centrifuged at 10,000 rev^{-1} for 5 min and the supernatant was recovered, 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 described by Zapata and Dufour (1992) slightly modified (Gil et al., 1999). 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 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 of strawberry fruits.

Soil sampling and analysis

Each year, at different plant phenological phases (T1: at transplanting time, 0 DAT; T2: first flowering, 50 DAT; T3: start of harvesting, 200 DAT; and T4: at the end of the crop cycle, 280 DAT), four elementary soil samples were taken from each plot by using an auger at depth of 0-30 cm and mixed to form a composite sample for each plot.

Over the cropping cycle, total mineral nitrogen (SMN) at T1 (SMN-T1), T2 (SMN-T2), T3 (SMN-T3) and T4 (SMN-T4) were determined as the sum of nitric (NO_3^--N) and ammonium nitrogen (NH_4^+-N). Fresh soil samples were sieved at 2mm and extracted by 2M KCl (1:10 w/v). Then NH_4^+-N was determined according to Krom (1980) and NO_3-N according to Henriksen and Selmer-Olsen (1970).

Every year, at the beginning of the experiment (T1) and at the end of the cropping cycle (T4), the soil samples were dried in oven at 105°C overnight to determine Total Organic Carbon (OC-T4) and total N (N-T4). Soil OC-T4 has been measured by using a LECO Carbon Analyzer. Soil N-T4 was determined by Kjeldahl method.

Nutrient budget and organic carbon input

N budget was evaluated following the criteria proposed by Watson et al.(2002) for the calculation of the surface input/output balance. Nutrient budget have been estimated accounting the inputs and outputs of N in each system. Total input was calculated by adding initial soil available nitrogen (SMN-T1 converted in kg of N per hectare) plus unit of nitrogen supplied by organic fertilizers, amendments and ASCs. Total output was calculated by adding the nitrogen up-taken by fruits and plant residues to the final soil available nitrogen (SMN-T4 converted in kg of N per hectare). The nitrogen budget resulted by output less input. According to Möller (2009), atmospheric N deposition, symbiotic N_2 fixation and gaseous losses via denitrification (N_2 and N_2O) were not included in the N budget, due to the difficulties in assessing reliable amounts of these inputs and losses.According to this calculation, the higher the budget the higher is the input that has not been removed from the systems (N surplus). While, the closer to zero is the budget, the more equilibrated is the system in the short term. Negative values of N budget indicates a depletion of soil N fertility (N deficit), not sustainable in the long run. Moreover, each year, in order to calculate the organic carbon input (OC-Input), carbon content of ASCs, organic fertilizers and amendments were multiplied by the correspondent biomasses per hectare values.

Statistical analysis

Univariate analysis of variance (ANOVA) on the whole dataset considering Year (Y) as random factor, and System (S) as fixed factor, was performed. Before analysis, the Levene Test was performed to verify the homogeneity of error variances. Mean comparison was carried out according to the Tukey Test, at $P \leq 0.05$ probability level. The elaboration was carried out by using STATISTICA (StatSoft, Inc. 2007, version 8.0). A principal components analysis (PCA) has been performed to describe the whole variability of the recorded fruits quality data at harvesting time throughout a multivariate methodology. The CANOCO 5 software has been used to elaborate an unconstrained PCA. Furthermore, in order to summarize the correlation among organic fertility management strategies, N budget, yield and quality of organic strawberries in the experimental conditions, a subset of supplementary variables has been reported on the PCA biplot. In particular, as main parameters discriminating the compared systems, OC-T4, SMN-T4 in kg of N per hectare, Ntot, C-input, N-budget and Yield parameters were used to explain the experimental variation of the quality traits based on the different organic production systems.

3.3 Results

Yield evaluation

In both years, strawberries were harvested according to fruit ripening. In 2013, harvesting season lasted 58 days, while in 2014 only 48 days. No significant difference was observed for yield in the S x Y interaction. On the other hand, significantly higher yields were detected in 2013 (30.3 Mg ha-1) compared to 2014 (28.9 Mg ha-1; $P \leq 0.05$), while no differences were observed for system factor (data not shown). The yields at any harvesting time, for each year, (in Mg ha-1) are reported in Fig. 1a, b. As for precocity of strawberry production, in the first year, SB yields at the first harvesting times (171 DAT and 179 DAT) were significantly higher than AM, while AC was characterised by an intermediate level of production (Fig. 1a). At 196 DAT, all systems reached a peak of production, with AM showing the significantly lowest value. While, during the last month of harvesting, AM systematically produced more than the other two systems, even if the difference was not statistically significant, recovering the production gap of the first harvesting times. In 2014, the yield differences among systems were never significant, being the peak of production reached at 206 DAT (Fig. 1b).

Table 1. Agroecological service crop (ASC) mixtures used before strawberry and their ecological functions. MIX1 and MIX2 have been cultivated before strawberry in the AGROMAN (AM) and AGROCOM(AC) systems, respectively.

	Botanical family included in the ACS mixtures (% by seeds weight)				**Method of termination**	**Expected Agro-ecological function of the mixtures**
	Polygonaceae	*Fabaceae*	*Boraginaceae*	*Brassicaceae*		
Mix 1 (AM)	*Fagopyrum esculentum* **36%**	*Trifolium resupinatum* *Trifolium alexandrinum* *Vigna sinensis* **56%**	*Phacelia tanacetifolia* **8%**	**0%**	Incorporated as dry biomass into the soil	Improve nutrients cycling
Mix 2 (AC)	*Fagopyrum esculentum* **36%**	*Dolichos lablab* **44%**	**0%**	*Raphanus sativus* *Eruca sativa* **20%**	Incorporated as fresh biomass into the soil	Suppress nematodes and pathogens
Agro-ecological services in literature	Nutrient cycling efficiency Weed suppression Beneficial insects attraction	N fixation Soil organic matter increase Soil and water quality	Beneficial insect attraction	Nitrogen losses decrease Nematode suppression Soil-borne disease reduction Soil and water quality Root growth increase		
(Reference)	(Clark, 2008).	(Snapp *et al.*, 2005)	(Bugg, 1991)	(Nett *et al.*, 2011)		

Table 2. Soil physical and chemical characteristics in May 2012 before greenhouse establishment. Means (Avg) and standard deviation (SD) values of 18 samples are reported.

Parameters	**Unit**	**Avg**	**SD**	**Method of analysis**
Stones and gravel >2mm	g kg^{-1}	212.61	33.23	
BD (Bulk Density)	g cm^{-3}	1.1	0.08	
Textural class		Loam		USDA
pH (H_2O)		7.9	0.06	Aqueous extract (1:2.5 w/v)
pH (CaCl)		7.6	0.05	CaCl extract
EC (Electrical Conductivity)	dS m^{-1}	0.4	0.02	Aqueous extract (1:2 w/v)
OC (Organic C)	g kg^{-1}	15.6	1.69	Walkley-Black
OM (Organic Matter)	g kg^{-1}	26.8	3.14	OM = OC x 1.724
TN (Total Nitrogen)	g kg^{-1}	1.3	0.15	Kjeldahl
C/N (OC / TN)		12	1.45	
P (available P)	mg kg^{-1}	25.1	8.33	P- Olsen
K (exchangeable K)	mg kg^{-1}	407.2	61.87	

Table 3. Organic amendments and commercial fertilizers supplied per each system.

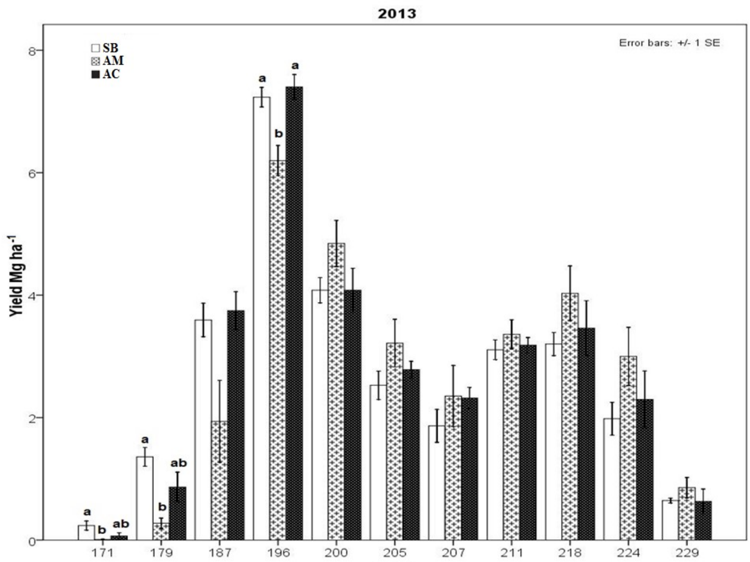
System	Amendments (Dry matter)	Commercial fertilizers based on Guano	Commercial fertilizers Based on Vegetal proteins[1]
	$Mg ha^{-1}$	$Mg ha^{-1}$	$kg N ha^{-1}$
AC	16 (*on-farm compost*)	0	5.4
AM	16 (*Cattle manure*)	0	9.5
SB	0	1.9	20.3

[1]liquid fertilizer (reported as $kg N ha^{-1}$ instead of $Mg ha^{-1}$); SB = SUBSTITUTION; AM = AGROMAN; AC = AGROCOM

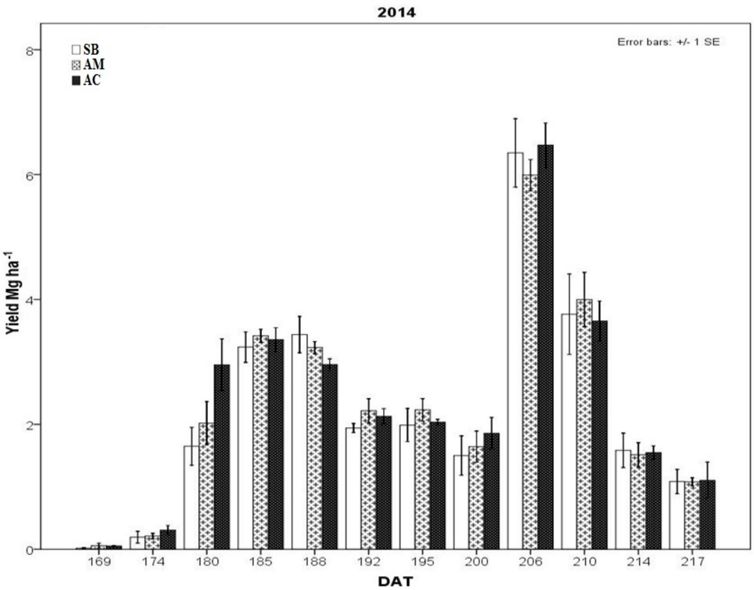
Table 4. Chemical characteristics of the amendments and of the commercial fertilizers supplied to the three systems.

Soil amendments and Fertilizers	TOC[1]	TN	P	K_2O	C/N	Sources and manufacturers
	$g kg^{-1}$					
Compost	260.5	27.0	6.09	16.06	9.7	*On-farm compost produced from plant residues (green compost) at MAIB experimental compost facility.*
Cattle manure	430.2	17.9	5.54	93.30	24	*Animal manure based on cattle manure from the organic husbandry 'La Querceta'*
Guano	320.0	60.0	150	30	5	*Italpollina S.p.A. (Italy).(Commercial name: Guanito)*
Vegetal hydrolised proteins	150.0	30.0	-	60	5	*Sugar-beet by products with vegetal hydrolysed protein. Serbios company (Commercial name: Kappabios)).*

Notes: [1] TOC = Total Organic Carbon; TN = Total Nitrogen; P = Total Phosphorus; K_2O = Total potassium; C/N = C/N ratio



a.

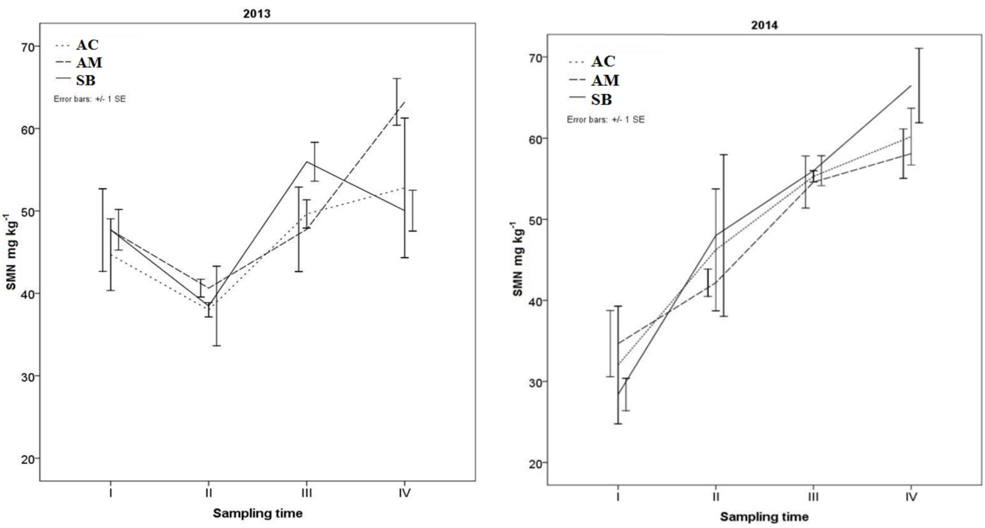


b.

Figure 1. Yield at different harvesting times in 2013 (a) and in 2014 (b). DAT: Days after transplanting. SB = SUBSTITUTION; AM = AGROMAN; AC = AGROCOM

Available soil mineral nitrogen and N budget

No significant differences for available SMN were recorded for S, Y and S x Y interaction. Either in 2013 and in 2014, available SMN showed a high content from transplanting to harvest for the three compared systems (Fig. 2a, b).



a. b.

Figure 2 a (2013) b (2014). Soil Mineral Nitrogen at T1, T2, T3,T4 (I = transplanting time; II = first flowering; III = start of harvesting; IV = end of the crop cycle). SB = SUBSTITUTION; AM = AGROMAN; AC = AGROCOM

The ANOVA results for N budget are reported in Table 5. No significant S x Y interaction was found for the tested parameters, while significant differences, due to the S factor, were observed for the Total N input and N budget. In particular, the AM and AC had significantly higher total N input (up to 169%) than SB, despite no differences among systems were observed for SMN-T1. Accordingly, the SB system showed the lowest N surplus (N budget), due to the absence of differences in Total N output among treatments. As far as the Y factor was concerned, the first experimental year was characterized by higher strawberry Nuptake (in terms of residues) and SMN-T4. It resulted in Total N output values higher in 2013 than in 2014. As a consequence, a significantly higher N surplus was observed in 2014 compared to the previous year (558.3 and 315.0 kg ha^{-1} of N, respectively).

Fruit quality

The effects of year (Y), system (S) and the Y x S interaction on strawberries quality attributes are reported in Tab. 6. As far as the Y factor is concerned, L*, a*, b*, Chroma, Hue°, pH, AA, DHA, VitC and TotPh showed significant differences. All parameters, with the exception of L*, showed higher values in 2014 than in 2013. On the other side, for S factor, no significant differences were observed for almost all the parameters, except pH, AA and VitC. In particular, pH was lower in SB (3.93) and AM (3.93) than in AC (4.13), while, for AA and VitC, AM showed the significantly highest values. The Y x S interaction was significant only for TA and TotPh (data not shown). In AM system, according to Tukey (0.05), TA was significantly higher in the first year (0.77 % citric ac. $100mL^{-1}$ of fruit juice) than in the second one (0.57 % citric ac.), while TotPh was significantly lower in 2013 (95 mg gallic ac. $100g^{-1}$ of fresh weight) than in 2014 (154 mg gallic ac. $100g^{-1}$ of fresh weight).

Principal component analysis

The Principal Component Analysis (PCA) summarized the variation of the quality trait composition at harvest (Fig. 3 and 4).

Table 5. Soil N surplus/deficit or N-budget (kg N ha-1) for the strawberry growing season of the whole experiment by systems and years.

kg ha^{-1}	**N Input**					**Total N input**	**N Output**			**Total N output**	**N-budget**
	On farm			*Off farm*							*Input - Output*
	SMN-T1	ASCs	Compost	Manure	Organic fertilizers		Fruit Nuptake	Residues Nuptake	SMN-T4		
Systems (S)[1]											
SB	188.4	-	-	-	135.4	323.8 b	25.9	89.0	122.2	237.0	86.8 b
AM	199.3	374.1	-	286.4	11.3	871.1 a	26.1	91.6	134.3	252.1	619.0 a
AC	189.2	228.3	432.0	-	5.4	854.9 a	27.6	94.0	129.2	250.8	604.1 a
Sig.	*n.s.*	-	-	-	-	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*
Year (Y)[2]											
2013	182.2	122.3	144.0	95.5	50.9	594.8	27.9	99.0 a	152.9 a	279.8 a	315.0 b
2014	202.5	279.3	144.0	95.5	50.5	771.7	25.2	84.0 b	104.2 b	213.4 b	558.3 a
Sig.	*n.s.*	-	-	-	-	*n.s.*	*n.s.*	*	**	***	*
Overall	192.3	200.8	144.0	95.5	50.7	683.3	26.5	91.5	128.6	246.6	436.6
S x Y	*n.s.*	-	-	-	-	*n.s.*	*n.s.*	*n.s.*	*n.s.*	***n.s.***	***n.s.***

Notes: 1 The mean values of the two years are reported by systems; 2 The mean values among the three systems are reported by year. The mean values followed by different letters are significantly different according to Tukey test for P ≤ 0.05. SB = SUBSTITUTION; AM = AGROMAN; AC = AGROCOM

Table 6 Effect of production systems on quality attributes of organic strawberries at harvest for the whole experiment by systems and years.

	L*	a*	b*	Chroma	Hue°	Firmness (%)	pH	TSS (%)	TA (%)	AA (mg 100 g^{-1})	DHA(mg 100 g^{-1})	Vit C (mg 100 g^{-1})	TotPh(mg 100 g^{-1} gallic ac.)
Systems (S)[1]													
SB	36.94	25.80	10.18	28.00	20.32	10.10	3.93 b	10.96	0.61	23.89 b	5.18	29.06 b	130.05
AM	35.27	26.68	9.48	28.64	17.81	9.90	3.93 b	9.57	0.67	31.13 a	6.03	37.16 a	124.43
AC	37.08	26.23	9.86	28.37	18.55	11.04	4.13 a	10.85	0.66	24.10 b	6.06	30.15 b	121.06
Sig.	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	**	*ns*	*ns*	**	*ns*	***	*ns*
Year (Y)[2]													
2013	39.33 a	21.91 b	4.99 b	22.66 b	12.43 b	10.04	3.91 b	10.44	0.68	23.74 b	2.76 b	26.50 b	111.42 b
2014	33.53 b	30.57 a	14.69 a	34.02 a	25.37 a	10.65	4.09 a	10.47	0.62	29.00 a	8.74 a	37.75 a	138.94 a
Sig.	***	***	***	***	***	*ns*	**	*ns*	*ns*	**	***	***	**
Overall	36.43	26.24	9.84	28.34	18.90	10.34	4.00	10.46	0.65	26.37	5.75	32.13	125.18
S x Y	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*	*

Notes: 1The mean values of the two years are reported by systems; 2 The mean values among the three systems are reported by year. The p-values of the ANOVA are represented by stars such as: * = p<0.05; **= p<0.01; ***=p<0.001 The mean values followed by different letters are significantly different according to Tukey test for P ≤ 0.05. SB = SUBSTITUTION; AM = AGROMAN; AC = AGROCOM

Starting from the 22 tested quality parameters, the first two components of PCA (PC1: X-axis and PC2: Y-axis) explained about the 83% of the global experiment variability (PC1: 67% and PC2: 16%). The distance among the observations in the scatter chart approximates the dissimilarity of their quality trait composition. This leads to observe that X-axis completely discriminated the two experimental years regardless of the organic system (Fig. 3).

In particular, the plots belonging to the 2013 (reported with label '13') scored in the right side of the biplot while the 2014 plots (reported with label '14') scored in the left side. Differences on the horizontal direction are mainly attributed to Firmness, TA, AA and DHA, characterizing samples of 2014, on the negative part, and to L* values in the positive part of the axes. Looking to PC2, the conventionalized organic system SB scored in the upper quadrants of the biplot, while both the agro-ecological systems, AC and AM, scored in the bottom ones, with the only exception of one AC13 and one AM14 observations. PC2 resulted positively correlated with fruit Hue° and Chroma, and negatively correlated with pH and TotPh, representing AC and AM. No differences were, in fact, observed between the two agro-ecological approaches for the analysed parameters.

This finding is clearly confirmed by the position of the system centroids (that are represented by the solid triangles in the Fig. 4). Fig. 4 shows the results of the PCA with agronomic parameters (OC-T4, OC-Input, Yield, N-T4, SMN-T4 and N budget) added as supplementary variables in order to represent the relations between the agronomic and the fruit quality parameters. It can be observed that Yield, OC-T4 and SMN-T4 resulted correlated with X-axis, explaining difference between the 2 years, whereas OC-Input and N-Budget correlated with Y-axis, differentiating AC and AM from SB.

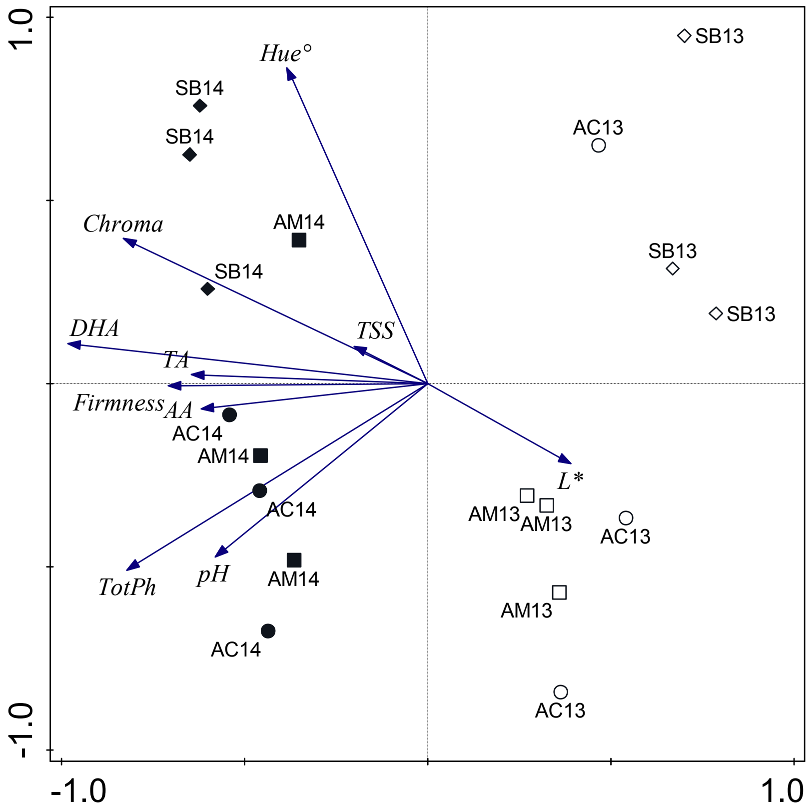


Figure 3. PCA Biplot (X-axes: 67%) and (Y-axes: 16%). Symbols: diamond for SB, circle for AC, square for AM; symbol area is filled for 2013 and it is left empty for 2014l. SB= SUBSTITUTION; AM=AGROMAN; AC=AGROCOM ; 13=1st year-field II (2013); 14=2nd year field I (2014). Quality traits legend: L*, Hue° and Chroma are the fruit colour attributes;TSS= total soluble solids in percentage;TA=Titratable acidity; pH= fruit pH; Vit C= Vitamic C; TotPh=Total Phenols of fruit;Firmness=% of fruit diameter deformation under pressure.

As it is possible to observe in Fig. 3, the biplot of the multivariate analysis presented the eigenvectors of AA and Phenols (TotPh) in the left side of the chart showing an increasing trend during the years. This is in correlation with the increasing of OC in the soil as showed in Fig. 4. Moreover, TotPh arrow is in the low part of the chart (Fig. 3) where agro-ecological systems plots are scattered.

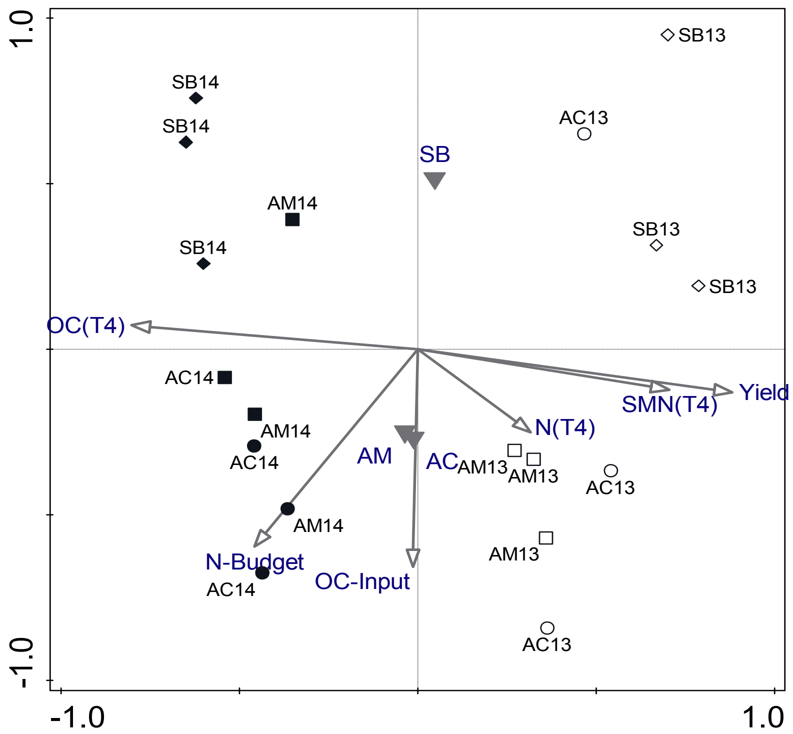


Figure 4. Plot scores PCA with supplementary variables. Symbols: the solid triangles are centroids (average score of each system in the first component); diamond for SUBSTITUTION (SB), circle for AGROCOM (AC), square for AGROMAN (AM); symbol area is filled for 2013 and it is left empty for 2014; 13=1st year-field II (2013); 14=2nd year field I (2014). Supplementary variables legend: at the end of strawberry production: OC(T4): soil organic carbon at T4; N(T4): soil total nitrogen, SMN(T4): soil mineral nitrogen; during the crop cycle: OC-input: Organic carbon input (from ASCs, organic amendments and fertilizers); N-budget: nitrogen budget; Yield: Strawberry cumulative yield in dry matter

3.4 Discussion

The three compared organic production systems did not differentiate significantly in terms of strawberry quantitative yield in both the experimental years. This result indicates that strawberry production was characterised by similar cumulative yields both in organic conventionalized (SB) and organic agro-ecological (AM, AC) systems, confirming the hypothesis that even more complex fertilization patterns can guarantee similar level of production of more simplified systems. Each year, the SMN trends during the whole crop cycles, from T1 to T4, were coherent with the observed yields (Fig. 1). In particular, in both years, similar SMN at transplanting (T1) indicated that the three different systems guaranteed the same available mineral N level at the beginning of the strawberry cycle. This goal was achieved despite the different origins and C/N ratios of the organic materials incorporated to soil (Table 4). During all the phenological phases, the synchronization of available soil mineral N with plant requirements was demonstrated in the compared systems (Fig. 2). On the other side, this cannot sufficiently explain the different timing of the beginning of harvesting, which is usually attributed to vegetative vigor, confirming the higher level of complexity of the fruit maturation physiology. As far as the strawberry quality was concerned, the results of the SMN seems to be not coherent with the total soluble solid values. Indeed, Wang and Millner (2009) observed that, the higher the SMN concentration, in comparison to the plant demand level, the lower the TSS of strawberry fruits. On the contrary, in our experiment, the overall mean value of total soluble solids (10.46 %) resulted higher than the values reported in literature (8.00 %) for the same variety transplanted in the same period of the year (Rahman, 2014). Moreover, the easier the soil nutrients are available for the roots uptake the higher the plant cells production is oriented toward the primary metabolism leading to big fruits, high protein content and low concentration of secondary metabolites (Gill et al., 2010). Among the latter, Vitamin C and total phenols have been considered for the present study. Similarly, Gòmez-Lòpez and del Amor (2013), in their study on sweet pepper, observed that the highest concentration of VitC was obtained for systems with organic amendments (horse and sheep manures).

In the present study the differences in strawberries quality between organic conventionalized and organic agro-ecological systems retrace the results of conventional/organic comparison studies reported in scientific literature. Despite the compared systems were all within the borders defined in the EU Regulatory framework for organic farming, the organic conventionalized system has presented strawberry with diverse characteristics in respect to organic agro-ecological system produce. Similar differences of the same nature, are reported among conventional and organic system. Studies of organic-conventional comparison (Amodio et al. 2007; Brandt et al., 2011; Jin et al., 2011; Oliveira et al., 2013) reported VitC content significantly higher in the organic than in conventional systems. Few published studies compared phenols values between organically and conventionally grown strawberries, finding higher phenols content in organic strawberry fruits than in conventional ones (Asami et al., 2003; Reganold et al., 2010). Similar considerations might concern the strawberry colour attributes. Multivariate analysis has shown high values of Hue° in SB fruits.

By assuming the same values of L*, the higher the Hue° value, the less red the strawberry fruit colour perception (Jouki and Dadasphour, 2012; Amal et al., 2010). In this respect, Charissa et al. (2013) reported that strawberry Hue° is inversely correlated to the anthocyanin fruits contents and it might be a suitable screening tool.

The input-output balance highlighted a higher N-surplus in the agro-ecologically managed organic systems than in the conventionalized one. Since the budgets do not provide information about the fate of any N-surplus (Watson et al., 2002), a more in depth direct and indirect evaluation of the different N forms in soil is needed. As a matter of fact, no significant differences were observed among systems in terms of SMN, along the entire strawberry cropping cycle. In particular, as pointed out above, either AM and AC, which incorporate in soil high quantities of organic materials, do not seem to immobilize SMN compared to SB (Fig. 2a, b). Moreover, the introduction of short cycle green manure in the rotation should, in the long run, reduces the risk of nutrient imbalances (mainly N/P and N/K) which is typical of intensive systems of production as reported by Voogt and co-authors (2011). Furthermore, nitrate leaching in the compared systems was measured and resulted negligible (data not reported), due to lack of rainfall (protected condition) and to accurate irrigation protocols. These results, in accordance with Ciaccia et al. (2015), put in evidence that the most part of the N-surplus was probably represented by organic N forms (whose sources were mainly ASCs, compost and manure). As a consequence, in our experiment, the N-surplus might be the basis of N long term fertility of the agro-ecological production systems, being available for subsequent crops in rotation (Tittarelli et al., 2014). On the other side, SB showed a more equilibrated N-budget respect to the other two systems, denoting the capability of sustaining the yield of the current crop (similar strawberry yield compared to AC and AM systems; Fig. 1a, b), but with no effect on N fertility in the long run. Moreover, the results of the PCA based on the fruit quality parameters, have been further described with the help of supplementary agronomic variables (Fig. 4). These parameters (OC-input, N-budget, OC-T4, SMN-T4, yield) were either calculated during the whole production cycle and analysed at the end of strawberry production. Results put in evidence that soil organic carbon clearly increases with the time (from right to left). Considering the Y-axis, which discriminate the three compared systems, the input of carbon (OC-input) and the surplus of nitrogen at the end of harvest (N-budget) are positively correlated to the agro-ecological systems (AC and AM).

So, it is possible to affirm that the differences in inputs, in terms of quantity and quality of organic matter added to soil, have been effective in improving both the soil properties in terms of organic carbon and the organic strawberry fruit quality of AM and AC.As expected, the two agro-ecological treatments did not differentiate in the period of time analysed. The lack of significant differences between AC and AM and the lack of parameters discriminating the two agro-ecological treatments put in evidence that, in the short term, total C input discriminates the treatments much more than the type of amendment applied to soil (compost or animal manure) and the mixtures of ASC utilised.

3.5 Conclusions

An agro-ecological approach to organic strawberry production in Mediterranean greenhouse is challenging from different point of views: i) quantity and quality of strawberry production, ii) soil fertility management and, more in general, iii) system sustainability. The results obtained with our research, demonstrated that the higher level of complexity of agro-ecological approaches, in comparison to a "conventionalized" organic one, does not reduce the yields expectation.

Meanwhile, nutrients crop requirements were guaranteed by all treatments during the whole crop cycle of strawberry, characterized by a harvesting period of around two months. The two agro-ecological treatments determined a surplus of organic N in soil, potentially available for the next crops in rotation, without the negative side effects of nitrate leaching. The implementation of systems of production, which determined an increase of soil organic nitrogen pool, is the basis for long term soil fertility management in Mediterranean organic greenhouse. From a quality production perspective, AM and AC allowed to obtain strawberry fruits similar to SB, but with better characteristics in terms of color and phenolic content which, for their influence on organoleptic properties and antioxidant effects, potentially have a strong market appeal. Differences between the two compared agro-ecological treatments were not significant in the framework of a biennial experiment.

On the basis of the results obtained so far, a complete analysis of the agronomic and productive performances of the different treatments and of their economic sustainability for the entire rotation would be desirable to confirm the findings observed for strawberry and for a more accurate evaluation of the compared systems of production.

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4. Quality and postharvest performance of organically-grown strawberry 'festival' under unheated tunnel[3]

Abstract

Presently, the same organic certification may obtained through very different systems of production. Organic techniques can rely on complex agro-ecosystem management or they can just replace chemical fertilizers and pesticides with allowed inputs. The aim of this research was to study the quality characteristics and their evolution during storage of organic strawberries produced by agroecological or conventionalized organic systems. Different organic soil fertility management strategies were compared under the same environmental conditions: i) a simplified organic production system based on organic commercial fertilizers (SUBST); ii) organic production system based on cover crops and animal manure amendment (AGROMAN), and iii) organic production system based on vegetal sources inputs like: cover crops and on-farm compost (AGROCOM). Strawberry fruits were sampled and stored in a cold room at 1°C under a continuous humidified air flow for 13 days. At 0, 3, 6 and 13 days after harvest respiration rate, morphological, physical, and sensorial characteristics, and nutritional quality attributes were monitored. At the time of harvest mean values for all measured parameters did not

[3] Ceglie, F. G., Piazzolla, F., Amodio, M. L., Mimiola, G., Colelli, G. (2014, October). Quality and postharvest performance of organically-grown strawberry 'festival' under unheated tunnel. Selected as oral presentation at the *ISHS: III International Symposium on Organic Greenhouse Horticulture, Izmir – 15.Apr.2016*.

show significant differences among treatments, while after 13 days of refrigerated storage hue angle, vitamin C content and sensorial scores (sweetness and off-flavours)were significantly higher for the organic strawberry grown in SUBST compared to all other systems. In conclusion, the production system did not influence the quality of 'Festival' strawberry cultivated in greenhouse under Mediterranean climate conditions at harvest but they significantly affected postharvest performances during 13 days of storage.

4.1 Introduction

Strawberry raw consumption represents a significant source of folate, vitamin C, polyphenols and other antioxidants (Gianpieri et al, 2012). According to some published literature, organic products may contain more of these compounds than conventional ones (Amodio et al., 2007; Brandt and Mølgaard, 2001) and this, together with the lower amount of chemical residues, was identified as the primary reason for purchasing organic food (Zanoli et al., 2004). In organic systems, many methods are used to maintain soil fertility; the soil incorporation of organic amendment and the green manuring of cover crops increases the soil organic matter input that slowly releases available nutrients; on the contrary mineral fertilizers, allowed only in conventional farming systems, are highly concentrated in nutrients that are directly available for the root uptake. However organic commercial fertilizers (included in the annex I of CE Reg.889/08), that are deeply used in organic agriculture, may mineralize much more nutrients per unit of time than the amendments thus resulting in a similar availability of nutrients to the one obtained with conventional techniques. The effects of organic farming fertility management on the quality of organic strawberry at harvest and during postharvest storage have not been deeply investigated in the literature. One published work is related to the comparison of conventionally and organic grown fruits at harvest and after freezing storage (Barbieri et al, 2015).

Reganold et al. (2008) linked the high quality of organic strawberry fruits with the high capability and stress resilience of organically managed soil. However, the organic certification may be obtained through very different systems of production. On one hand organic techniques can rely on holistic agro-ecosystem management, on the other hand, they can just replace chemical fertilizers and pesticides with allowed inputs. The former is based on agro-ecological practices while the latter is based on input substitution. In particular, the case of organic greenhouse production is a clear example of this dualism. The high intensive production level recorded in greenhouse systems requires high availability of nutrients to sustain the crop growth; as a consequence organic growers simplify the crop rotation, exclude cover crops and utilize easily soluble organic fertilizers. It is the "conventionalization" process of the organic farming (Goldberger, 2011) which may be summarized in the development of organic farms that are implementing practices that may not be sustainable but that are not explicitly excluded by the organic standards (Padel et al., 2007). The research hypothesis is that the different approach in the soil fertility management of organic greenhouse production may affect the organic strawberry quality at harvest and during post-harvest storage. In this respect, the main objective of this experiment was to compare the effect of agroecological and substitution approaches on quality and postharvest performances of strawberries.

4.2 Materials and methods

Experimental site and design

The experiment took place at the experimental farm of the Mediterranean Agronomic Institute in Bari (Apulia region, Italy, 41°.0536 N; 16°.8766 E) from June 2013to May 2014under an unheated plastic tunnel. The organic farming systems under comparison were: i)SUBST, an organic input substitution widely adopted in organic production system, especially in greenhouse horticulture, which mimics conventional agriculture by substituting conventional agrochemicals with allowable organic products; ii) AGROMAN, characterised by the use of a cover crop mixture (based on legume species) and mature manure (from organic husbandry); and iii) AGROCOM, which uses a different cover crop mixture (mainly based on brassica species) and compost produced on-farm (at the experimental composting facility of MAIB). The three organic farming systems were randomly assigned in three replications for a total of 9 plots (3.0 x 4.0 m) in a complete randomised block design. Further information about the experimental design have been already reported in previous works (Mihreteab et al., 2014 and Ceglie et al., 2015).

Cultural practices and plant material

In June 2013seeds of cover crop mixtures were sown in AGROMAN and AGROCOM plots, while in the SUBST system the soil was kept bare for the period of cover crop production. After 50 days the cover crop biomass was incorporated into the soil together with: 16 t ha^{-1}(dry matter) of compost in the AGROCOM system and the same rate of manure in AGROMAN plots. Organic commercial fertiliser (Guanito: NPK= 6,15,2%, Italpollina s.r.l., Italy) was incorporated at a rate of 1.6 t ha^{-1} before strawberry transplantation, regularly sugar beet molasses (Kappabios, a soluble organic commercial fertiliser: NPK = 3,0,6%, Serbios s.r.l., Italy) was supplied every week at 4.5 gm^{-2}, in fertigation. Kappabios was also applied in AGROCOM and in AGROMAN only in case of early nutrient deficiency symptom. 'Festival' strawberry seedlings were transplanted on late September; their production started in March and lasted until May. In April, from each plot, all the ripe fruits were hand harvested and quickly delivered to the postharvest laboratory at the University of Foggia (Apulia region, Italy).

Physical and chemical quality characteristics

Fruit diameter was taken with a digital caliper. Colour was measured on two sides of the fruit, using a colorimeter (CM-2600d, , Minolta, Osaka, Japan) in the CIE L*a*b* mode, and then hue angle, Hue°=arctan b^{*2}/a^{*2}, and chromaticity, Chroma=SQRT($a^{*2} + b^{*2}$), were calculated.

Firmness was determined on ten strawberry fruits for each replicate as percentage of diameter deformation under a force applied of 5N (kg m s^{-2}) between two parallel plates using an Universal Testing Machine (INSTRON 3343 Norwood, MA, US).

Each fruit was squeezed and the obtained juice drops were used for direct readings of total soluble solids (TSS%) content with a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan). Total acidity (TA) and pH were measured on 5 g of squeezed juice for each replicate, using an automatic titrator (TitroMatic CRISON, HACH LANGE, Spain) with 0.1 mol L^{-1} NaOH solution to pH 8.1 and reported as percentage of citric acid per 100mL. Vitamin C (Vit-C),L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), content was determined as described by Zapata and Dufour (1992) with some modifications (Gil et al., 1999).

Total phenols were determined according to the method of Singleton and Rossi (1965).The content of total phenols was calculated on the basis of gallic acid calibration curve and expressed as mg gallic acid $kg^{-1}$of fresh weight.

Weight loss and respiration rate

Strawberry were subsequently stored in a cold room at 1°C; about 1.5 kg of fruits were placed in three perforated clam shell trays which were positioned into individual containers (5 L) one for each treatment-replicate. Each container was connected to a continuous flow of humidified air.

During the storage, at 3, 6 and 13 days, the weight loss and the fruit respiration rate (mL $CO_2 kg^{-1} h^{-1}$) were monitored. .Carbon dioxide concentration was measured using using the dynamic system (Kader, 2002a) by injecting a sample of the output gas into a chromatograph (Shimadzu 17A, Kyoto, Japan) equipped with a TCD detector (200 °C) and then referred to the weight of the sample, nd for the air flow as measured by a flowmeter (ADM100 Agilent Technologies, Inc. 2850 Centerville Road Wilmington, US).

Sensorial attributes

A blinded panel test was performed by a group of six trained panellists to assess fruits appearance, colour, acidity, aroma, sweetness on a qualitative scale 5 to 1 (5= excellent, 3=fair, limit of marketability, and 1=very poor). Off-flavour were evaluated from 1 to 5 where 1 denoted no off-flavour and 5 denoted very intense off-flavours development.

Statistical analysis

All the physical and chemical determinations were performed in three replications at harvest and at 3, 6, and 14 days of storage.The significant level of production systems, time of conservation and their interaction were evaluated on the measured parameters by two ways ANOVA (STATISTICA 8.0, Statsoft inc., Tulsa, OK, US) e post-hoc Tukey ($p \leq 0.05$). Per each systems, the aggregate average of the data recorded for the whole period of the experiment were evaluated only in the event that the attributes didn't present significant interaction between factors.

4.3 Results and discussion

The experiment compared strawberry fruits quality at harvest and during cold storage of three organic farming systems: two "agro-ecological"(AGROCOM and

AGROMAN) and one "conventionalized" (SUBST). Production system did not significantly affect quality attributes of organic strawberry fruits at harvest.

Average fruits diameter ranged between 26.1 and 28.1 mm (data not shown). TSS (%) resulted among 9.6 and 11.1 that is higher than the values reported in literature for the same cultivar (Rahman et al., 2014).

Vitamin C mean values ranged from 34.9 to 42.0 mg 100 g^{-1}FW (Figure 1) slightly lower than the values in the nutrient database for standard reference (USDA 2010). Finally, total phenols ranged from 130.8 to 153.6 mg gallic a. 100 g^{-1} FW (data not shown). The absence of significant differences in the quality attributes at harvest indicates that the three investigated soil fertility management strategies didn't affect the organic strawberries quality.

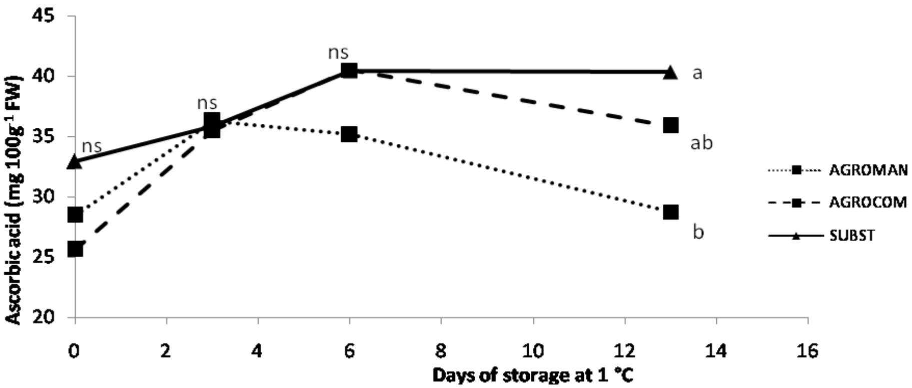

Figure 1.Evolution of ascorbic acid content in 'Festival' strawberries from different organic production systems during storage at 1 °C. Per each day of storage, different letters indicate significant differences, ns: non-significant ($p<0.05$ Tukey).

Fruits L*, pH, TSS and total phenols did not vary significantly neither for the systems of production, nor during the storage and nor for the interaction of both the effects according to the ANOVA table (Table1).

Table 1. ANOVA table of *p-values* for the effect of production systems, days of storage and their interaction on quality parameters of organic strawberry 'Festival'

Strawberry parameters		FARMING SYSTEM (SY)	TIME of STORAGE (ST)	SY x ST
Weight loss	(%)	ns	ns	ns
Respiratory activity	(mL CO^2 kg^{-1} h^{-1})	*		
L*		ns	ns	ns
a*		ns	**	ns
b*		*	***	**
Chroma		ns	***	*
Hue°		*	***	**
Firmness	(%)	*	***	ns
pH		ns	ns	ns
TSS	(%)	ns	ns	ns
TA	(% citric ac.)	ns	*	ns
AA	(mg 100 g^{-1} FW)	*	***	ns
DHA	(mg 100 g^{-1} FW)	ns	***	ns
Vit C	(mg 100 g^{-1} FW)	ns	*	ns
Total phenols	(mg 100 g^{-1} gallic ac.)	ns	ns	ns
Appearance		ns	***	ns
Colour		ns	***	ns
Aroma		ns	***	ns
Acidity		ns	*	ns
Sweetness		*	***	*
Off-flavours		*	*	*

ns: non significant or significant at: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$

The production systems did significantly affect only b*, Hue°, Firmness and AA. The storage significantly affected a*, b*, Chroma, Hue°, Firmness, TA, Vit-C, DHA and AA. Moreover, the factors interaction affected b*, Chroma and Hue°.

In particular, other than time factor, the effect of the different production systems resulted in Ascorbic Acid significantly higher in SUBST than in AGROCOM (Figure 1). Per each system of production, during the whole period of storage all the parameters presented similar trend with the only exceptions of b* (Figure 2), and Hue (Figure 3).

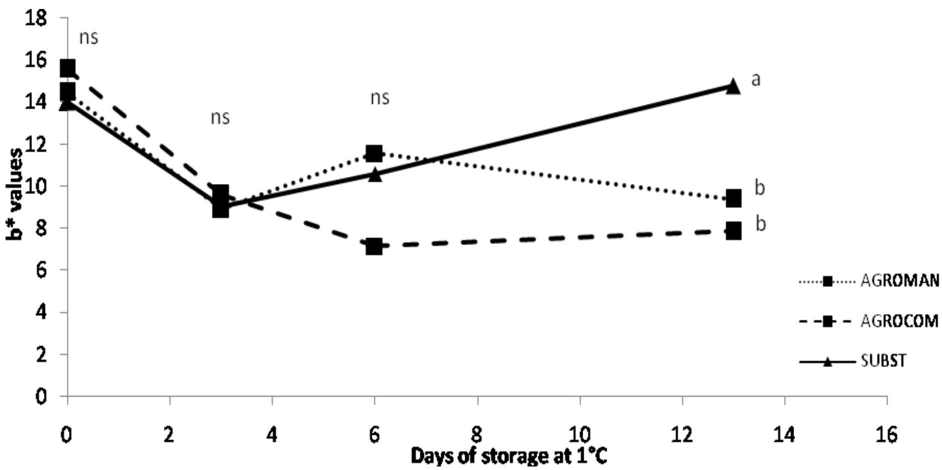

Figure 2. Evolution of b* value in 'Festival' strawberries from different organic production systems during storage at 1 °C. Per each day of storage, different letters indicate significant differences, ns indicates non significance ($p<0.05$ Tukey).

As for b*at 13 days it was observed that resulted significantly higher in SUBST (14.8) in comparison to AGROMAN (8.9) and AGORCOM (7.2), while Hue° resulted significantly lower in AGROCOM (14.5) than in AGROMAN (17.9) and SUBST (8.9). Having similar L* values, the higher the Hue° values, the less red is the fruit colour perception (Amal et al., 2010).

The firmness in AGROMAN increased during storage from 10.5% to 15.8% indicating fruits softening during postharvest.

Further effects of the storage on the strawberries quality have been observed on the AA (Figure 1). In AGROCOM it slightly increased after harvest (25.62 mg 100 g^{-1}) until the end of the experiment (35.88 mg 100 g^{-1}). While, DHA acid in SUBST decreased significantly from 3 (11.21 mg 100 g^{-1}) to 13 (4.78 mg 100 g^{-1}) days of storage. The same situation, has been observed in AGROMAN where DHA decreased from 11.46 mg 100 g^{-1} at 3 days to 3.59 mg 100 g^{-1} at 6 day, even if it has increased again at 13 day to reach 7.21 mg 100 g^{-1}. This difference in the rate of biological deterioration might be due to the different physiological activity as observed for the respiration rate, much more than external factors being the samples stored in the same conditions of temperature, air flow and relative humidity (Kader, 2002b).

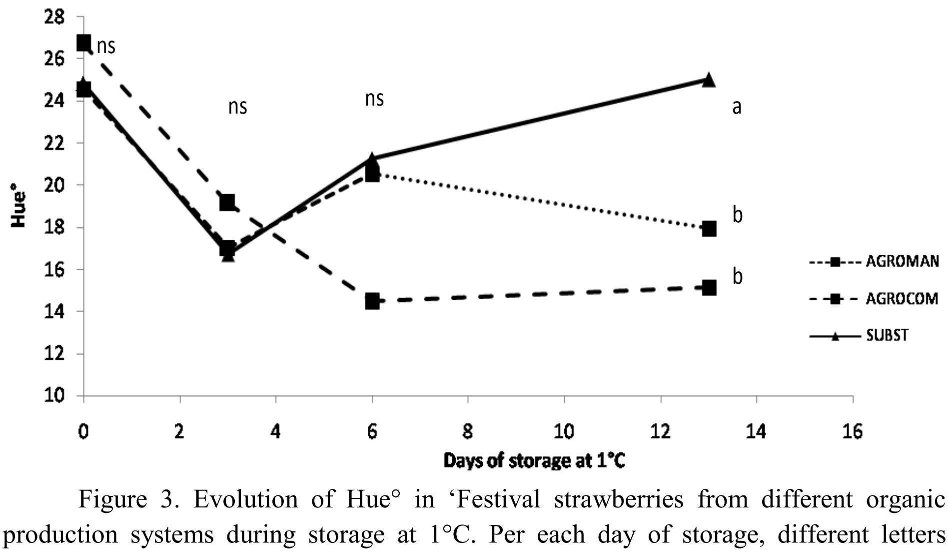

Figure 3. Evolution of Hue° in ‘Festival strawberries from different organic production systems during storage at 1°C. Per each day of storage, different letters indicates significantly different means; ns indicates non significance ($p<0.05$, Tukey).

At 3 and 6 days of storage, in fact, the respiration rates resulted significantly lower for SUBST (7.6±0.5 and 8.3±0.3 mL CO_2 kg^{-1} h^{-1} respectively) than for both

AGROMAN (9.5±0.9 and 11.7±1.7 mL CO_2 kg^{-1} h^{-1} respectively) and AGROCOM (12.3±1.5 and 12.6±1.8 mL CO_2 kg^{-1} h^{-1} respectively). It is worth noting that SUBST showed a lower respiratory activity which may have led to a better preservation of the quality at harvest (Figure 4). After harvest the quality loss of the fruits compositions cannot be maintained, respiration and transpiration lead to biological deterioration of the products (Brosnan and Sun, 2001).

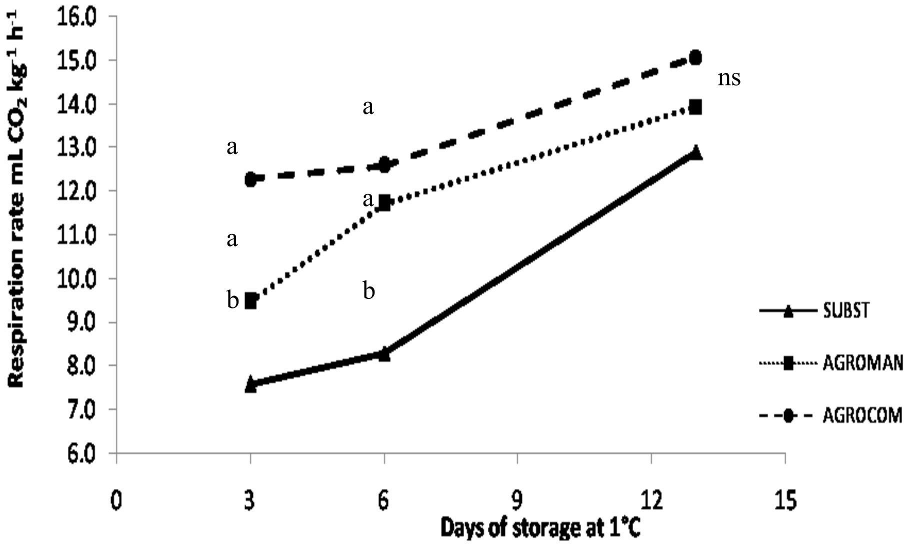

Figure 4. Evolution of respiration rate in 'Festival' strawberries from different organic production systems during storage at 1 °C. Per each day of storage, different letters indicate significant differences, ns indicates non significance ($p<0.05$ Tukey).

The low respiratory activity recorded in SUBST at 3 and 6 day of storage may be the cause of the lower rate of decline of quality parameters as reported for Ascorbic acid and for the sensorial attributes at 13 days of storage (Figure 5).

No differences in weight loss were observed among treatment, resulting between 0.5 and 1.6% of the initial weight.

As for sensorial attributes, at harvest, panel test scores for all the organically grown strawberries showed similar results (Figure 5a), as well as after 3 days (Figure 5b). At 6 days of storage, the panellists were able to appreciate significantly higher sweetness and aroma in AGROMAN than in the AGROCOM (Figure 5c).

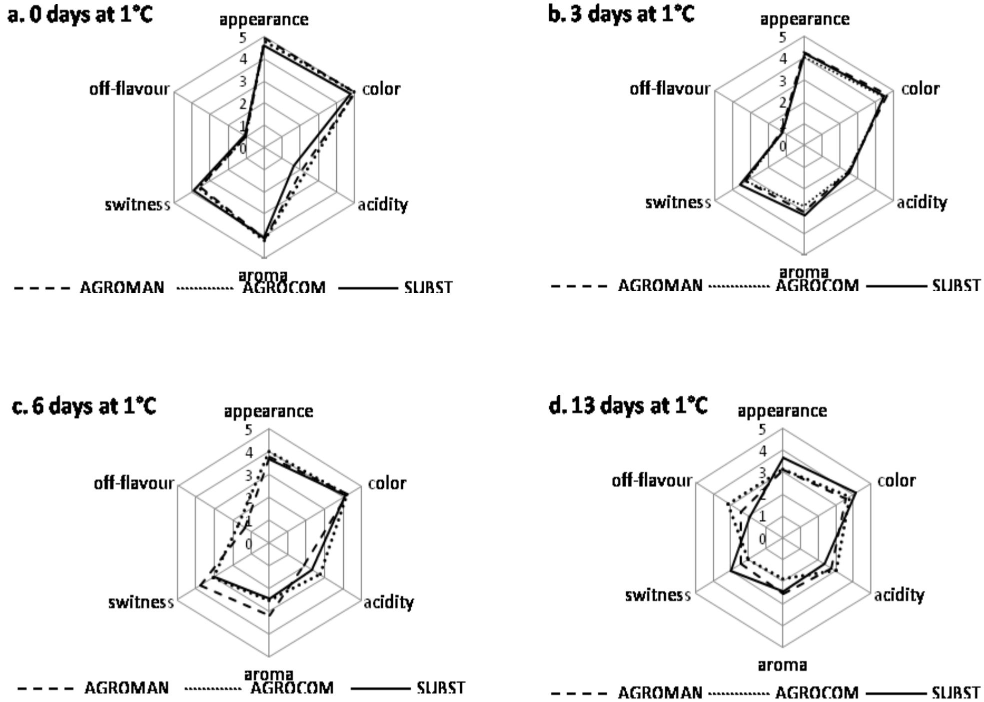

Figure 5. – Sensorial test scores at 0 (a), 3 (b), 6 (c), 13 (d) days of storage (1°C).

While at the 13 days, SUBST obtained 3±0.7 sweetness mean scores which was higher than 2.3±0.5 of AGROMAN and of 2±0.7 in AGROCOM. Furthermore, SUBST recorded lower off-flavours scores (1.8±0.6) than AGRCOMAN (2.4±0.6) and AGROCOM (3.1±0.6) (Figure 5d). It resulted, at the end of the experiment, the best organic system able to maintain the sensorial characteristics for the longest period of storage.

4.4 Conclusions

In conclusion, the production system affected the post-harvest quality parameters of organic 'Festival' strawberries cultivated in unheated tunnel under Mediterranean climate conditions.

Different soil fertility management practices production didn't result in different quality at the harvest of the organic strawberry fruits but affected the maintenance of the produce quality during the storage period.

The conventionalized systems SUBST better maintained strawberry fruits quality in cold air storage conditions.

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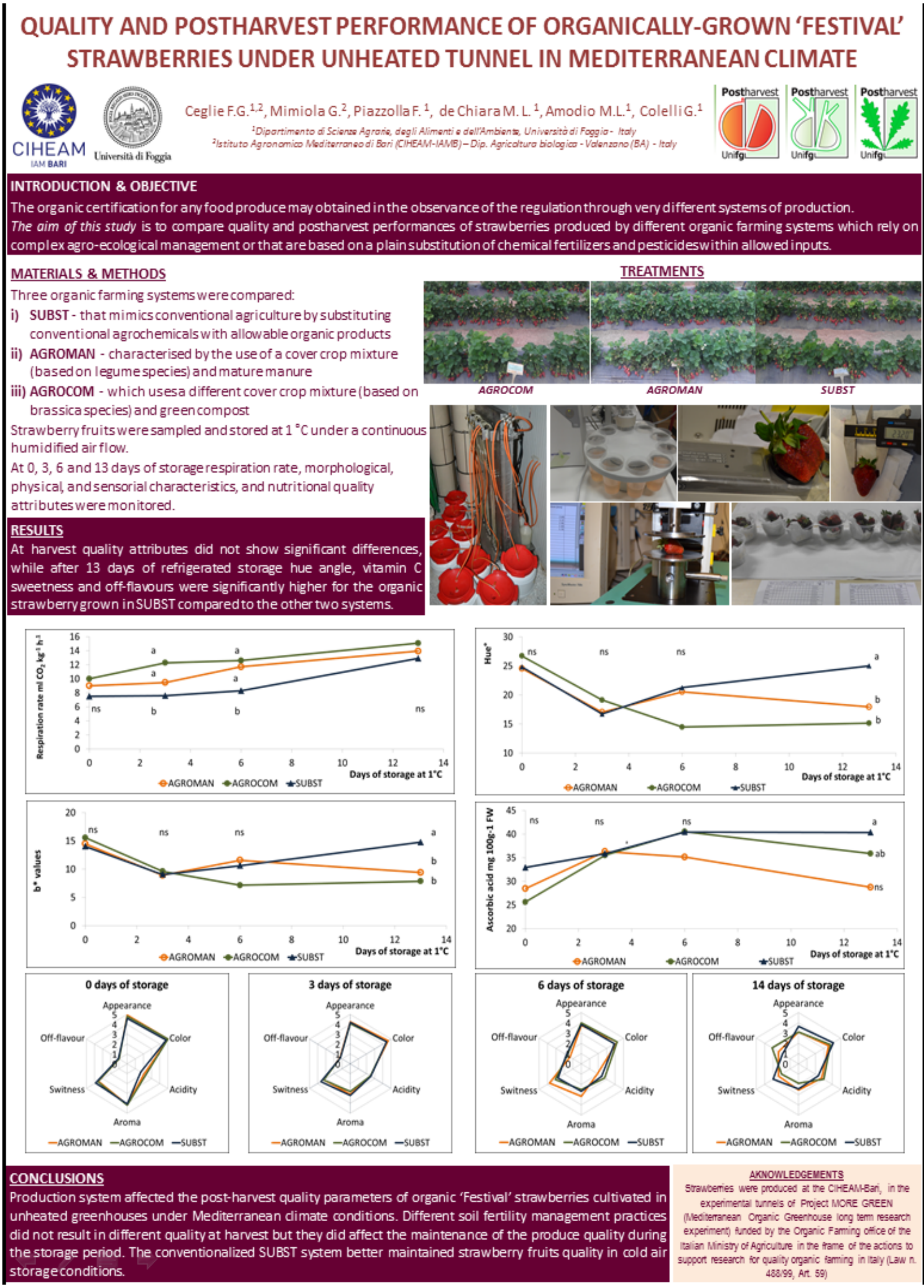
QUALITY AND POSTHARVEST PERFORMANCE OF ORGANICALLY-GROWN 'FESTIVAL' STRAWBERRIES UNDER UNHEATED TUNNEL IN MEDITERRANEAN CLIMATE
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INTRODUCTION & OBJECTIVE
The organic certification for any food produce may obtained in the observance of the regulation through very different systems of production.
The aim of this study is to compare quality and postharvest performances of strawberries produced by different organic farming systems which rely on complex agro-ecological management or that are based on a plain substitution of chemical fertilizers and pesticides within allowed inputs.
MATERIALS & METHODS
Three organic farming systems were compared:
i) SUBST - that mimics conventional agriculture by substituting conventional agrochemicals with allowable organic products
ii) AGROMAN - characterised by the use of a cover crop mixture (based on legume species) and mature manure
iii) AGROCOM - which uses a different cover crop mixture (based on brassica species) and green compost
Strawberry fruits were sampled and stored at 1 °C under a continuous humidified air flow.
At 0, 3, 6 and 13 days of storage respiration rate, morphological, physical, and sensorial characteristics, and nutritional quality attributes were monitored.
TREATMENTS
AGROCOM
AGROMAN
SUBST
RESULTS
At harvest quality attributes did not show significant differences, while after 13 days of refrigerated storage hue angle, vitamin C sweetness and off-flavours were significantly higher for the organic strawberry grown in SUBST compared to the other two systems.
Respiration rate ml CO2 kg-1 h-1
Days of storage at 1°C
Hue°
b* values
Ascorbic acid mg 100g-1 FW
AGROMAN
AGROCOM
SUBST
0 days of storage
3 days of storage
6 days of storage
14 days of storage
Appearance
Color
Acidity
Aroma
Switness
Off-flavour
CONCLUSIONS
Production system affected the post-harvest quality parameters of organic 'Festival' strawberries cultivated in unheated greenhouses under Mediterranean climate conditions. Different soil fertility management practices did not result in different quality at harvest but they did affect the maintenance of the produce quality during the storage period. The conventionalized SUBST system better maintained strawberry fruits quality in cold air storage conditions.
AKNOWLEDGEMENTS
Strawberries were produced at the CIHEAM-Bari, in the experimental tunnels of Project MORE GREEN (Mediterranean Organic Greenhouse long term research experiment) funded by the Organic Farming office of the Italian Ministry of Agriculture in the frame of the actions to support research for quality organic farming in Italy (Law n. 488/99, Art. 59)

5. Quality and postharvest performance of organically-grown tomato (*Lycopersicon esculentum* L.' Marmande') under unheated tunnel in Mediterranean climate[4]

Abstract

The organic production method includes a defined list of technical tools that are allowed in the EU Regulation and which can lead to different product quality. Different approaches to the fertility management of tomato organic production were compared in relation to the quality at harvest and during storage. Three organic farming systems were implemented in an experimental greenhouse: i) a simplified organic production system based on organic commercial fertilizers (SUBST); ii) organic production system based on animal manure amendment and cover crops (AGROMAN), and iii) organic production system based on green manuring of cover crops and on-farm compost amendment (AGROCOM). At harvest, breaker and pink tomatoes were sampled from each system. Breaker tomatoes were subsequently stored in a cold room at 15°C and ripened up to pink stage within 10 days. The effect of the fertilization systems was studied comparing breaker tomatoes after 10 days of storage and pink tomato ripened on plant and after storage. Yield, morphological indexes, dry matter, firmness, and composition were evaluated. The three systems produced comparable total (58.87±5.4 t ha^{-1}), and marketable (48.19 ± 5.1 t ha^{-1}) yields. AGROMAN fruits were larger in comparison

[4] Ceglie, F. G., Muhadri, L., Piazzolla, F., Martinez-Hernandez, G. B., Amodio, M. L., & Colelli, G. (2014, October). Quality and postharvest performance of organically-grown tomato (*Lycopersicon esculentum* L.'Marmande') under unheated tunnel in mediterranean climate. In *V International Conference Postharvest Unlimited 1079* (pp. 487-494).

to the other systems. AGROCOM system led to lower firmness, acidity and carotenoids than SUBST system and also showed the lowest dry matter. At harvest, SUBST tomatoes at the breaker stage showed higher vitamin C than AGROCOM, while this difference was not statistically significant after ripening. Pink tomatoes ripened on plant showed higher soluble solids content than pink tomatoes ripened in storage; moreover for AGROCOM and SUBST the carotenoids content was higher when ripened on the plant. In conclusion, in complex systems (AGROMAN and AGROCOM), it is possible to synchronise the mineralisation rates of organic amendments and green manure with the needs of the plants, and to obtain similar tomatoes yields and quality of simpler and less sustainable systems (SUBST).

5.1 Introduction

Tomato is a major components of human diets and, especially for its raw consumption all year round, it represents a significant source of folate, vitamin C, polyphenols and other antioxidants (Charanjeet *et al.*, 2004; Willcox *et al.*, 2003). Organic products may contain more of these compounds than conventional ones (Amodio *et al.*, 2007; Brandt and Mølgaard, 2001) and this has been identified as the primary reason for purchasing organic food, much more than environmental concern (Zanoli and Bahr, 2004). Preharvest and postharvest factors such as cultivar, ripening stage at harvest, storage conditions and agricultural practices affect the composition of fresh tomatoes (Dumas et al., 2003). In organic systems, many methods are used to maintain soil fertility; the soil incorporation of organic amendment and the green manuring of cover crops increases the soil organic matter input that slowly releases available nutrients for roots uptake. On the other hand organic commercial fertilizers (included in the annex I of the UE Reg.889/08) may mineralize much more nutrients than the amendments per unit of time but they provide low organic matter input. The effects on the quality at harvest and during postharvest of the organic products, of those different soil fertility managements strategies in organic farming are not been deeply investigated in the literature (Williams, 2002). A field trial was established in the spring of 2013 at the Mediterranean Agronomic Institute of Bari (MAIB), Valenzano (Italy) to implement different fertility management strategies in organic greenhouse tomato (*Solanum lycopersicum* L. cv. marmande) production. The main objective of the experiment was to investigate the quality, composition and postharvest performances of the organic tomatoes growth under different pre-harvest conditions.

5.2 Materials and Methods

The field experiment took place at the experimental farm of the Mediterranean Agronomic Institute in Bari (Apulia region, Italy, 41°.0536 N; 16°.8766 E) from June to December 2012 under an unheated plastic tunnel. The organic farming systems under comparison were: i) organic input substitution (SUBST), a widely adopted organic production system, especially in greenhouse horticulture, which mimics conventional agriculture by substituting conventional agrochemicals with allowable organic products; ii) AGROMAN, characterised by the use of a cover crop mixture (MIX1) and mature manure (from organic husbandry); and iii) AGROCOM, which uses a cover crop mixture (MIX2) and compost produced on-farm (at the experimental composting facility of MAIB). The cover crop MIX1 was based on species able to produce quite a lot biomass per unit of time (*Pennisetum glaucum*; *Fagopyrum esculentum* and *Vicia* spp.); while the MIX2 was made by *Vigna sinensis*, *Fagopyrum esculentum*, *Phacelia tanacetifolia* and *Trifolium* spp. Experiments were conducted in a 300 m^2 (7.5 x 40.0 m) unheated high tunnel (EUROPROGRESS s.r.l., Italy) made of a galvanised steel frame covered with ethylene vinyl acetate sheets. The tunnel was divided down the length into two fields (I: tomato-green bean and II: strawberry). A complete randomised block design was implemented with three blocks (13.3 x 3.2 m) per field. The three organic farming systems were randomly assigned to the three blocks of each field for a total of 18 plots (3.0 x 4.0 m). The 1st June 2013, in AGROMAN plots, manure was incorporated at about 20 cm depth by a two wheel tiller, at a rate of 16 t ha^{-1}, before hand-sowing the MIX1 seeds at a rate of 75 kg ha^{-1} on 6th June. In AGROCOM plots, MIX2 was sown in the same way and at the same time as MIX1. In the SUBST system, soil was kept bare for the period of cover crop production and organic commercial fertiliser (Guanito: NPK= 6 15 2%, Italpollina s.r.l., Italy) was incorporated at a rate of 1.6 t ha^{-1} before tomato transplantation, plus sugar beet molasses (Kappabios, a soluble organic commercial fertiliser: NPK = 3, 0, 6%, Serbios s.r.l., Italy) was supplied every week at 4.5 g m^{-2}, in fertigation. A black polyethylene film was used to prevent weeds. After 60 days of cover crops (MIX1) cultivation, in the AGROMAN system, a roller-crimper was used to flatten the biomass to cover the soil surface as a dead mulch. By using this technique, the cover crops created a weed-barrier and provided a small amount of nutrients through decomposition during tomato cultivation (Canali et al., 2013). Then, after tomato transplantation, Kappabios was applied every two weeks by fertigation to meet nutrient requirements of the crop. After 50 days of cover crops (MIX2) cultivation, in the AGROCOM system, compost was incorporated into the soil at a rate of 16 t ha^{-1}, during the green manuring of the MIX2 biomass. Then, Kappabios was applied at 30 and 60 days after transplantation (DAT). Table 1 shows the total N, P and K applied to each farming system from the different sources. Tomato seedlings of cultivar 'Marmande' were transplanted into prepared plots on August the 21^{st}. Each plot had three rows with a total of 39 tomato plants per plot. Per each plot, all the tomato fruits that were mature at least at the breaker stage were hand harvested on December 13, and total and marketable yields were determined and evaluated according to local market standards. The tomatoes were quickly delivered at the postharvest laboratory of the University of Foggia. The maturity stages of the fruits were evaluated according to the Agricultural Marketing Service and Vegetable Division (United States, Fresh Products, 1997). The breaker stage (when the fruit presents a break in colour from green to red and not exceeding the 10% of the surface) has been selected for storage trial because it is the most requested at the market. In addition, at harvest ten breakers and ten pink tomatoes have been sampled for each plot (representing an experimental replication). Fruit dimension (two diameters and the height) were taken with a digital calipers. Firmness was determined on ten tomato fruits for each replicate and defined as the deformation (in mm) under a force of 5N (kg m s^{-2}) between two parallel plates using an INSTRON Universal Testing Machine (model 3343 United Stated & Canada ©).

Table 1. Total N, P and K supplied by cover crop, manure, compost and/or organic commercial fertilisers in each organic greenhouse horticulture production systems on tomato crop in 2013.

				Organic commercial fertilisers[2]		Total
System[1]	Cover crops	Compost	Manure	Guanito	Kappabios	kg ha^{-1}
N (kg ha^{-1})						
SUBST	-	-	-	95.7	10.8	106.5
AGROCOM	119.4	432.0	-	-	2.7	554.2
AGROMAN	255.5	-	286.4	-	5.4	547.3
P (kg ha^{-1})						
SUBST	-	-	-	101.5	-	101.5
AGROCOM	6.9	97.4	-	-	-	104.3
AGROMAN	19.7	-	88.6	-	-	108.3
K (kg ha^{-1})						
SUBST	-	-	-	31.1	21.6	52.7
AGROCOM	123.9	257.0	-	-	5.4	386.3
AGROMAN	284.7	-	149.3	-	10.8	444.8

[1]SUBST - no cover crop; 100% off-farm organic fertilisers; AGROMAN - soil-incorporated animal manure + cover crop (MIX1) flattened into natural mulch before tomato transplantation; AGROCOM - soil-incorporated on-farm produced compost and green manuring of cover crop (MIX2).

[2]Guanito (Italpollina s.r.l. Italy) + Kappabios (Serbios s.r.l. Italy)

Relative deformation (%) was calculated as the relative percentage of the fruit deformation. Each fruit was squeezed and the obtained juice drops were used for direct readings of total soluble solid (TSS) content (%) with a digital pocket refractometer (Atago PR32-Palette, Tokyo, Japan). Total acidity (TA) and pH were measured on 10 g of squeezed juice for each replicate, using an automatic titrator (TitroMatic CRISON 1S, Spain) with 0.1 mol L^{-1} NaOH solution to pH 8.1 and reported as percentage of citric acid. Vitamin C content was assessed on 5 g of homogenised tomato fruit tissue as L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA) contents as described by Zapata and Dufour (1992) with some modifications (Gil et al., 1999). Total vitamin C was reported as the sum of both DHAA and AA contents. Total phenol content (TPC) was determined on 5 g of tomato fruit tissue according to the method of Amodio et al. (2014) and results were expressed as mg of gallic acid equivalent/100 g fw. For the determination of the carotenoids, the chlorophyll a, b and its total contents, the extraction was made by keeping the samples into 15 mL methanol for 24 h at 20 °C in darkness. The extract was then spectrophotometrically read at 653, 666 and 470 nm, an equation reported by Wellburn (1994) was used to calculate the pigment contents and expressed as mg/100 g fw. Breaker tomatoes were subsequently stored in a cold room at 15°C, the fruits were kept separated in individual containers (15 L), one for each treatment-replicate connected to a continuous flow of humidified air. The respiration rate (mL CO_2 kg^{-1} h^{-1}) was measured using the dynamic system (Kader, 2002) at the first and the tenth day of conservation. The pink tomatoes at harvest weren't stored so their respiration rate wasn't evaluated. CO_2 concentration was measured using a Shimadzu gas chromatograph (model 17A) equipped with a TCD detector (200 °C). Separation of CO_2 was achieved on a Carboxen 1006 plot (30 m X 0.53 mm, Supelco, Bellefonte, PA, USA) which was then corrected for the weight of the sample, to the elapsed time and to the air flow that was measured by flux meter (model ADM100 Agilent Technologies, Inc. 2850 Centerville Road Wilmington, DE 19808-1610 USA). All the analyses were also repeated after ten days of storage at 15 °C. The measured parameters were statistically evaluated by ANOVA (XLSTAT 7.5.2) to assess the effects of the fertilization systems on breaker and pink tomatoes at harvest, breaker tomatoes after ten days of storage, pink tomato ripened on plant and after storage. The significant differences among groups of mean values were evaluated by post-hoc test (Tukey $p<0.05$). Finally a principal components analysis has been performed in order to better describe the whole variability of the recorded data throughout a multivariate methodology.

5.3 Results and discussion

In the different systems, the tomato yields were comparable. No significant ($p<0.05$) differences were observed both in terms of total production (AGROMAN: 53.05 ± 4.0 t ha^{-1}; SUSBT: 63.73 ±11.5 t ha^{-1}; AGROCOM: 59.84 ± 3.9 t ha^{-1}) and marketable yield (AGROMAN: 42.62 ± 2.3 t ha^{-1}; SUBST: 52.46 ± 9.5 t ha^{-1}; AGROCOM: 49.49 ± 2.2 t ha^{-1}). The agroecological systems of organic production (AGROCOM and AGROMAN which include cover crops mixtures and organic amendments) were able to obtain yields similar to SUBST which is based only on easily soluble organic fertilizers. At harvest time, fruit height was about 5.3 cm and fruits diameters resulted in the range 7.6-8.5 cm with any significant (p<0.05) difference among systems. AGROMAN tomatoes had significantly higher mean weight (183.6 g) than both SUBST (170.3 g) and AGROCOM (167.5 g). Dry matter in AGROCOM fruits resulted significantly (p<0.05) lower than AGROMAN (Table 2). Tomato chemical composition is reported for breaker and pink tomatoes analyzed at harvest (Tables 2 and 3, respectively) and breaker tomatoes which were stored for ten days at 15°C (Table 4). Breaker tomatoes ripened up to pink stage during storage. The different fertility management strategies and the maturity stage significantly affected the organic tomatoes quality and postharvest storage. As far as system of productions is concerned, the fruits analyzed at harvest (both breaker and pink samples) presented significantly (p<0.05) lower chlorophyll and higher carotenoid content in the AGROCOM than the AGROMAN system (Tables 2,3). Those differences among the systems was no longer measured after storage. The respiration rate at breaker stage in AGROCOM (8.0 ml CO_2 kg^{-1} h^{-1}) was significantly lower in comparison to SUBST (9.3 ml CO_2 kg^{-1} h^{-1}); after 10 days at 15°C, it increased (from 5 to 10%) with no differences among the systems. Furthermore, significant (p<0.05) lower DHAA content resulted in AGROCOM respect to SUBST, while total vitamin C didn’t show significant differences. Accordingly, also phenols at breaker stage were significantly lower in AGROCOM (68.3 mg/100g) compared with AGROMAN (260.9 mg/100g^{-}). Lycopene and β-carotene are mainly responsible for the red coloration on tomato skin (Kotíková et al., 2011). Tomatoes showed lower firmness, TA and chlorophylls contents at the pink maturity stage than those corresponding to the breaker stage. A comprehensive description of all the results by using the principal component analysis is shown in Figure 1.

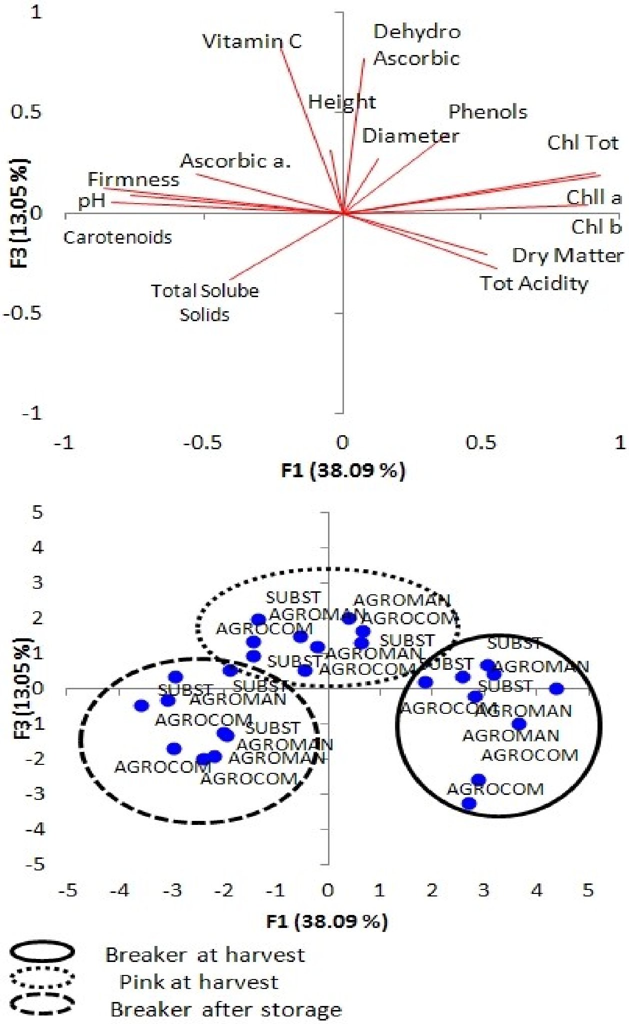

Figure 1. Principal components analysis on quality attributes of breaker (full line oval) and pink (dot line oval) tomatoes at harvest and of breaker tomato after storage (broken line oval). The first three components (eigenvalues): F1, F2, F3 explains about the 70.0 % of the total dataset variabilty. The quality variables (a) and the observations (b) of organic tomato fruits are represented in the cartesian diagram with F1, on the x-axis, and F3, on the y-axis. The oval shapes are used to cluster the observation at similar fruit maturity level and in the conservation conditions.

The first two components (F1 and F2) accounted for 58.3% of the total variability recorded. A third component has been considered in order to reach about the 70% of the whole dataset variability. The first component (F1) was positively correlated with the chlorophylls and negatively correlated with carotenoids content, pH, firmness. This component has been reported on the x-axis of the chart (Fig.1) and it was able to separate clearly the data into three groups which were, from right to left, the breaker tomatoes at harvest, the pink tomatoes at harvest and the breaker tomato after storage. The second component (F2) increased with the increase of fruits diameter and height, and was negatively correlated with soluble solids content and vitamin C. The third component (F3), on the y-axis versus F1 in Figure 1, resulted in positive correlations with vitamin C, dehydroascorbic acid and phenols content. Inside each cluster of maturity and cold storage which are grouped in an oval area, SUBST and AGROMAN observations were distributed close each other. On the other hand, AGROCOM observations resulted, with only one exception, in the negative part of F3 component. On the basis of the explained correlations of the fruits performance parameters and chemical characteristics, the observations distributed in the upper quadrants of the PCA chart showed the best results in terms of fruits size (diameter and height), vitamin C and phenol contents which are well known antioxidant compounds. While, in the third quadrant (at low-left) are distributed the fruits with a less acidic pH value and with higher sugar and carotenoid contents. This graphical representation of collected data allowed a more immediate evaluation first of the differences among the maturity stage and conservation of the fruits and then on the effect of the pre-harvest condition on the quality parameters and the postharvest performances of the organic tomatoes.

Table.2 – Quality parameters of breaker tomatoes at harvest

Breaker	SUBST		AGROMAN		AGROCOM	
Respiration (ml CO_2 $kg^{-1}h^{-1}$)	**9.3**	**a**	**8.2**	**ab**	**8.0**	**b**
AVG Fruit weight (g)	**170.3**	**b**	**167.5**	**a**	**183.6**	**b**
Diameter min (cm)	7.7		7.9		7.6	
Diameter max (cm)	8.5		8.3		8.2	
Height (cm)	5.3		5.4		5.3	
Firmness (%)	8.4		7.7		6.8	
Dry Matter (%)	**5.2**	**ab**	**5.5**	**a**	**5.0**	**b**
pH	4.1		4.1		4.0	
TSS (%)	3.9		3.9		3.8	
TA (%)	0.71		0.68		0.7	
AA (mg $100g^{-1}$)	4.0		4.4		4.4	
DHA (mg/100g of FW)	**4.3**	**a**	**2.0**	**ab**	**1.0**	
Vit-C (mg $100g^{-1}$)	8.3		6.4		5.4	
Chlorophyll a (mg $100g^{-1}$)	**4.8**	**ab**	**6.8**	**a**	**4.1**	
Chlorophyll b (mg $100g^{-1}$)	**0.5**	**b**	**0.7**	**a**	**0.5**	**b**
Total chlorophyll (mg $100g^{-1}$)	**5.3**	**ab**	**7.5**	**a**	**4.6**	**b**
Carotenoids (mg $100g^{-1}$)	0.6		0.5		0.4	
Total Phenols (mg gallic ac. $100g^{-1}$)	**185.2**	**ab**	**260.9**	**a**	**68.3**	**b**

Values with different letters on the same row different significantly ($p<0.05$).

Table.3 - Quality parameters of pink tomatoes at harvest.

Pink at harvest	SUBST	AGROMAN	AGROCOM
AVG Fruit weight (g)	164.5	181.7	186.6
Diameter min (cm)	7.5	7.5	8.0
Diameter max (cm)	8.2	8.2	8.7
Height (cm)	5.2	5.2	5.3
Firmness - Relative Deformation (%)	12.7	12.0	11.6
Dry Matter (%)	4.9	4.9	4.3
pH	4.2	4.3	4.3
Total soluble solid - TSS (%)	4.2	4.0	4.3
Total Acidity (TA) (%)	0.66	0.56	0.58
Ascorbic acid (mg $100g^{-1}$)	4.6	5.5	5.5
Dehydroascorbic acid (mg $100g^{-1}$)	**4.0 a**	**1.1 ab**	**0.0 b**
Vitamic C (mg $100g^{-1}$)	8.6	6.6	5.5
Chlorolphyll a (mg 100 g^{-1})	0.8	0.6	0. 7
Chlorophyll b (mg 100 g^{-1})	0.1	0.1	0.0
Chlorophyll tot (mg 100 g^{-1})	0.7	0.7	0.7
Carotenoids (mg 100 g^{-1})	**1.2 a**	**0.9 b**	**0.9**
Total Phenols (mg gallic ac. $100g^{-1}$)	112.8	55.4	70.2

Mean values with different letters on the same row different significantly (p<0.05 Tukey test).

Table.4 – Quality Parameters of breakers tomatoes after storage (10 days at 15°C)

Breakers after storage (pink stage)	SUBST		AGROMAN		AGROCOM	
Respiration rate (ml CO_2 kg^{-1} h^{-1})	**9.8**	**a**	**8.8**	**b**	**9.0**	**ab**
Fruit mean weight (g)	169.4		162.8		182.5	
Diameter min (cm)	7.9		8.0		8.0	
Diameter max (cm)	8.2		8.4		8.6	
Height (cm)	5.6		5.3		5.4	
Firmness-Relative Deformation (%)	0.115		0.103		0.100	
Dry Matter (%)	4.9		4.9		4.3	
pH	4.3		4.2		4.2	
Total soluble solid - TSS (%)	3.8		3.6		4.0	
Total Acidity (TA) (%)	0.54		0.52		0.61	
Ascorbic acid (mg 100 g^{-1})	5.1		4.9		5.3	
Dehydroascorbic acid (mg 100^{-1})	4.1		3.5		3.6	
Vitamic C (mg 100 g^{-1})	9.2		8.3		8.8	
Chlorolphyll a (mg 100 g^{-1})	2.8		3.5		3.0	
Chlorophyll b (mg 100 g^{-1})	0.3		0.2		0.3	
Chlorophyll tot (mg 100 g^{-1})	3.1		3.3		3.3	
Carotenoids (mg $100g^{-1}$)	0.8		0.8		0.8	
Total Phenols (mg gallic ac. 100 g^{-1})	220.3		193.5		186.2	

Mean values with different letters on the same row different significantly ($p<0.05$ Tukey test).

5.4 Conclusions

The agro-ecological organic system (AGROCOM and AGROMAN) produced the same tomato yield as that recorded in the organic conventionalised system (SUBST). In terms of tomato fruits quality and during the postharvest storage, those tomatoes produced only by using off-farm inputs (SUBST) showed comparable size and quality parameters in comparison to those produced by using on-farm input and agroecological practices. This demonstrates that even in a more complex system (which includes cover crop mixtures and organic amendments), it is possible to synchronise the mineralisation rates of organic amendments and green manure with the needs of the plants, and to obtain similar yields and quality to the ones obtained using simpler and less sustainable systems (SUBST). In particularly, among the agroecological systems, AGROMAN provided higher yield quantity and better maintained fruit quality. Further studies are needed to confirm the results obtained for tomato cv. Marmande for other genotypes and under different climatic conditions.

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Quality and postharvest performance of organically grown tomato (*Lycopersicon esculentum* L. cv. Marmande) under unheated tunnel in Mediterranean climate

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Introduction

The organic production method includes a defined list of technical tools that are allowed in the EU Regulation and which can lead to different product quality. Different approaches to the fertility management of tomato organic production were compared in relation to the quality at harvest and during storage.

Materials e methods

Three organic farming systems were implemented in an experimental greenhouse

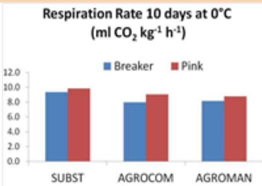
- ✓ SUBST: a simplified organic production system based on organic commercial fertilizers;
- ✓ AGROMAN: organic production system based on animal manure amendment and cover crops;
- ✓ AGROCOM: organic production system based on cover crops and on-farm compost.

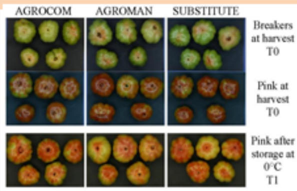
The effect of the fertilization systems was studied comparing breaker and pink tomatoes at harvest; breaker tomatoes after 10 days of storage; pink tomato ripened on plant and after storage.

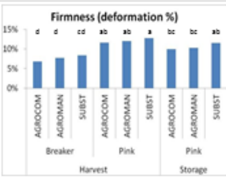
At harvest, breaker and pink tomatoes were sampled from each system. Breaker tomatoes were stored in a cold room at 15°C and ripened up to pink stage within 10 days. Yield, morphological indexes, dry matter (DM), firmness, and composition were evaluated.

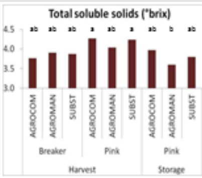
Results

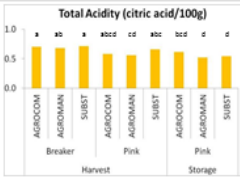
The three systems produced comparable total (58.9±5.4 t ha^{-1}), and marketable (48.2±5.1 t ha^{-1}) yields. SUBST fruits were smaller in comparison to the other systems. AGROCOM system led to lower firmness, acidity and carotenoids than SUBST system and also showed the lowest DM. At harvest, SUBST tomatoes at the breaker stage showed higher vitamin C than AGROCOM, while this difference was not statistically significant after ripening. Pink tomatoes ripened on plant showed higher soluble solids content than pink tomatoes ripened in storage; moreover for AGROCOM and SUBST the carotenoids content was higher when ripened on the plant.

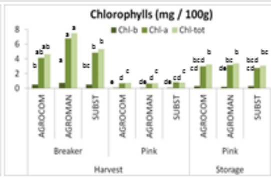


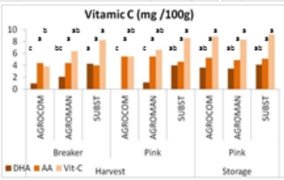


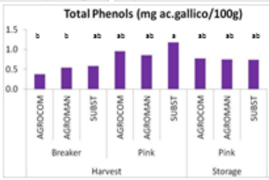


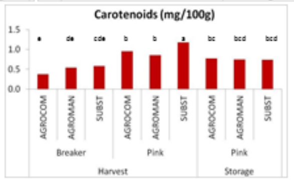












Mean values with different letters on the same series differ significantly (P<0.05, Tukey)

Conclusion

Tomatoes produced only by using off-farm input (SUBST) showed comparable size and quality parameters in comparison to the ones produced by using on-farm input and agroecological practices. In particularly, among the agroecological systems, AGROMAN has best maintained yield quantity and quality.

Acknowledgements

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6. Effect of different organic agronomic techniques and modified atmosphere packaging on the quality of organic lamb's lettuce

Abstract

The production techniques contribute in defining the quality characteristics of fresh fruits and vegetables at harvest but a limited amount of researches investigated whereas the production system may affect the postharvest evolution of the initial quality. This research focused on the effect of organic production systems on initial quality and postharvest performances of lamb's lettuce leaves stored in air or under modified atmosphere at refrigerated temperature. Different organic soil fertility management strategies were compared under the same environmental conditions: i) a simplified organic production system based on organic commercial fertilizers to recovery crop uptake (SB); ii) organic production system based on organic matter amendment mainly supplied by animal manure (AM), and iii) organic production system based on organic matter amendment supplied by vegetal sources inputs (AC). Fully developed lamb's lettuce leaves were harvested. Fifty grams of products (in three replications per each systems and time of storage) were packed into perforated (AIR) or sealed (MAP) bags. The bags were stored in a cold room at 4 °C for 11 days. Daily for each bag, oxygen and carbon dioxide partial pressure were monitored. At 0, 4, 7 and 11 days after harvest, weight loss, color (L*, a*, b*, Chroma and Hue°), firmness and chemical parameters (pH, total soluble solids, titratable acidity, ascorbic and dehydro ascorbic acids, antioxidant

activity and phenols) were analyzed. Finally, appearance and odor scores were subjectively evaluated after opening the bags. At harvest, an accumulation of phenols and dehydroascorbic acid in the system with the lowest initial supply of organic amendment (namely SB) was observed. A moderate stress for nutrient starvation has been suggested as possible explanation for this effect. In fact, the AM and AC systems preserved the highest rate of ascorbate pool in reduced form as a result of the fertility management strategy with a long term perspective, based on organic matter rich amendment. Although differences were minimal, AM and AC showed less quality changes over time than SB, whereas regarding the effect of packaging AIR conditions succeeded to maintain the initial quality attributes for a longer period in comparison to the modified atmosphere package that, at 11 day despite the production system, developed off-odors under the threshold of acceptability. Mainly the initial differences among the production systems were canceled or attenuated during in postharvest stage.

6.1 Introduction

Lamb's lettuce, appreciated for its sweet taste and soft texture, is growing in shear in the market of leafy vegetable especially for its nutritional value and for its status of healthy vegetables (Fontana et al., 2003). It is among the most requested baby leaves commercialized in the Italian market (Beghi et al., 2016). Lambs lettuce (*Valerianella locusta* L.), also known as corn salad, is an annual plant which belongs to the Valerianaceae family and it grows wild in the Nantes region of France which is also specialized in the lamb's lettuce production (Péron and Rees, 1998). It presents six-seven pairs of opposite leaves in form of rosette that might be harvested either including roots or as cut leaves. Some papers described the effect of organic amendments on lamb's lettuce quality, but generally literature about the effect of organic practice on quality of lambs lettuce is scarce. It is reported that the use of green waste compost increased phenol content in comparison with mineral fertilization of lamb's lettuce leaves (Kolton and Baran, 2008); another study reported a leaf nitrate content 10% lower when compost treatment was compared with the mineral fertilizer (Rooster, 1998). The lower nitrate content of the organic produce in respect to the conventional productions has been widely reported in literature, especially for leafy vegetables (Woese et al., 1997; Köpke, 2003; Lairon 2011); this has been accepted even in the much prudential review studies which compare organic and conventional produce (Bourn & Prescott 2002). Furthermore, high rate of mineral nitrogen increases nitrate and simultaneously decreases ascorbic acid concentration (Lee and Kader, 2000). Basically, mineral fertilizers are nearly total soluble nutrients, while, organic fertilizers and amendments shall be firstly mineralized to make nutrients available for the plant uptake. The nutrients mineralization relies on fertilizer characteristics, dosage and time of the application, and also on biotic and abiotic environmental conditions. It means that the synchronization between plant demand and nutrient availability is quite challenging in organic farming and it might lead to moderate plant stress due to nutrient starvation. Plant development and yield are greatly affected by environmental stresses that reallocate resources from primary to secondary metabolism (Cheynier et al. 2013) with a direct effect on the quality of the organic produce which should be considered also in relation to postharvest quality. Lamb's lettuce is primarily utilized as ready-to-eat salad, alone or as ingredient in the salad mix; in the packaging the leaves maintain a very small section on the petiole which is therefore exposed to oxidation as for rocket leaves (Egea-Gilabert at al., 2009; González et al., 2004). Postharvest quality management of lamb's lettuce leaves is limited by the yellowing of the leaves; color changes are caused by the chlorophyll and carotenoids degradation which increases during storage (Ferrante and Maggiore 2007). Retention of a dark-green color, with no yellowing or spots on the leaves, and texture are important for the perception of freshness by consumers and influence their choice (Dinnella et al., 2014; Ferrante et al., 2004). Appropriate packaging of green leafy vegetables delays senescence and yellowing (Kalio 2008, Kader et al., 1989). Generally, salad leaves are washed, packaged in modified atmosphere (MAP) with trays wrapped in plastic film to avoid physical damage and to prevent leaves wilting for the loss of water (Løkke et al., 2013; Cantwell et al., 1998). Modified atmosphere packaging (MAP) is a common technique used to extend the shelf-life of fresh or minimally processed products and it is a widely used to commercialize washed or unwashed lamb's lettuce. Passive MAP does not require any forced change in the gas concentration of the air in the package; the gas composition will inevitably change due to produce respiration and film permeability (Sivertsvik et al. 2002) and is applied as alternative to active atmosphere for baby and adult leaves which are less sensitive to browning compared to cut leaves. The appropriate package and a correct management of the temperature during storage preserve the quality at harvest, however the initial quality of the raw material depends on many pre-harvest factors related to pedoclimatic conditions and agronomic techniques. This research aims to compare the quality characteristics of organic lamb's lettuce produced by different organic systems, and to study their evolution during storage in passive modified atmosphere packages.

6.2 Materials and Methods

In March 2015, 50 seeds of lamb's lettuce (*Valerianella locusta* L. 'seme grosso d'Olanda') per square meter were sowed under unheated tunnel at the experimental farm of Mediterranean Agronomic Institute of Bari (Puglia region, Italy). Lamb's lettuce have been included as last crop in the organically managed vegetable crop rotation started in 2012 with a sequence of strawberries, tomato, green bean, cucumber, lamb's lettuce. Different organic soil fertility management strategies were compared under the same environmental conditions: i) a simplified organic production system based on organic commercial fertilizers (SB); ii) organic production system based on animal manure amendment (AM), and iii) organic production system totally based on vegetal sources inputs (AC). SB relies on short term approach to soil fertility management aiming to recovery the nutrient removed by the uptake per each crop of the rotation; while both AM and AC rely on a long term fertility management which aims to maintain or enrich the initial soil fertility level that may provide nutrient to the crops by mineralization of soil organic matter. Before the lamb's lettuce sowing, the soil mineral nitrogen was analysed per each production system to take into account the nitrogen available at the end of the previous crop (data not reported). In this way it is possible to calculate the nutrient that shall be supplied at each system to obtain similar starting conditions for lamb's lettuce. In this respect, mature manure (Biorex – Italpollina, Italy - N:2.8; P_2O_5:3; K_2O:2) was used in SB (20 kg N/ha) and in AM (100 kg N/ha), whereas neem cake (Radisana – Consigli dell'esperto s.r.l., Roma, Italy – N:3) was used in AC (70 kg N/ha). A pre-sowed blanket of biodegradable tissue (produced by Virens, Padova, Italy) was used to prevent weed competition and to obtain uniform plant density. Fully developed lamb's lettuce leaves were harvested 40 days after emergence. Samples of 50 g of products (in three replications per each systems and time of storage) were packed into perforated (AIR) or sealed (MAP) polypropylene bags of 280 cm^2 (OTR = 1800 cm^3 m^2 d^{-1}, WVTR = 6 g m^2 d^{-1}).

The product samples were stored in cold room at 4 °C for 11 days. Oxygen and carbon dioxide partial pressure were daily monitored. At 0, 4, 7 and 11 days after harvest, weight loss, color (L*,a*,b*, Chroma and Hue°), firmness, sensorial and chemical parameters were monitored.

Total soluble solids, titratable acidity, ascorbic and dehydro ascorbic acids, were analyzed on fresh samples, whereas antioxidant activity and total phenol content were analysed on frozen samples.

Packaging gas composition

Oxygen and CO_2 concentrations inside the packages were monitored with a gas analyser WITT Mapy 4.0 (Witten, Germany). Test probe of gas analyser was inserted into each package through an adhesive rubber septum to prevent air leaking from the package.

Sensorial analysis

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

Off-odors were scored on a 5 to 1 scale, where 1 = no off-odors, 2 = slightly off-odors, 3 = moderate off-odors (limit of marketability), 4 = strong off-odors and 5 = very strong off-odors, sulfur compounds.

Color

Color was measured using a Spectral scanner (DV SRL, Italia), equipped with a Spectral Imaging spectrometer V10 type (400-1000nm, 25µm slit, resolution 5nm).

One scan per replicate was acquired, at a speed of 3 mm/s in a dark room with a stabilized halogen light source (150W). On each leaf, a region of interest (ROI) corresponding to the maximum inscribed rectangle was manually selected. The instrument software allowed to automatically measure the mean value of L*, a*, b* (CIE L*a*b* color scale) of the ROI , elaborating the reflectance value of each pixel. $Hue° = \tan^{-1}\frac{b*}{a*}$ and $Chroma = \sqrt{a*^2 + b*^2}$ were calculated from L*, a* and b* values.

Firmness

Cut shearing force (N) was evaluated on 5 individual leaves per each replicate with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.) equipped with a miniature Kramer Shear cell (0.025X0.025 m of size) with 5 blades (HDP-MK05) which were moving at a speed of 2 mm s^{-1}.

Chemical analysis

Per each replicate, 5 grams of leaf tissues were squeezed to obtain juice drops which were used for direct readings of total soluble solids percentage (TSS), by a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan) while 2 g of juice were used for pH and TA measurements using an automatic titrator (TitroMatic CRISON, Barcelona -Spain) with 0.1 mol L^{-1}NaOH solution up to pH 8.1. TA was reported as percentage of citric acid per 100mL.

Vitamin C was determined using 5 grams of fresh tissue, which was homogenized with 10 mL of MeOH/H2O (5:95) plus citric acid (21 g L−1) with EDTA (0.5 g L−1). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata et al. (1992), with some modifications. The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20µL were analyzed with an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 v/v) containing 5mM cetrimide and 50mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min-1. AA and DHA contents were expressed as mg of ascorbic or dehydroascorbic acid per kg of fresh weight (mg kg^{-1}).

To determine the total phenol content, 5 grams of lamb's lettuce leaves were homogenized with an Ultraturrax (IKA, T18 Basic; Wimington, NC, USA) in methanol:water solution (80:20, 2 mmol L−1 in sodium fluoride) for 1 min. The homogenate was then centrifuged at 5 °C and 9000 rpm for 10 min. Total phenolic content (TPC) was determined by Folin-Ciocalteu assay (Singleton and Rossi, 1965).

Then, a corrected TPC value (TPC-AA) was obtained by subtracting the contribution of Ascorbic Acid content at the F-C- assay, as suggested by Brat et al. (2006) and Isabelle et al. (2010). The total phenol content was expressed as mg of gallic acid equivalent (GAE) 100^{-1} g fresh weight. It was found that 1 mg of AA had a reducing activity of 0.872 mg GAE, therefore AA content in the extract was multiplied by 0.872 and the result was subtracted from the TPC previously obtained to calculate TPC-AA. Similar approach was reported in literature (Sánchez-Rangel et al., 2013) by using the clorogenic acid equivalent.

Antioxidant assay was performed following the procedure with DPPH described by Brand-Williams et al. (1995) with minor modifications. The diluted sample, 50 μL, was pipetted into 0.950 mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after overnight incubation using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). Trolox was used as a standard and the antioxidant activity was reported in mg of Trolox equivalents per 100 g of fresh weight (mg TE 100 g^{-1} FW).

Statistical analysis

All data represent the mean of three replicates for each treatment and storage day. Data were subjected to analysis of variance for time of storage; significant differences among storage time were evaluated by Tukey's honest significance difference test ($p<0.05$) using STATISTICA 8.0 software.

6.3 Results and discussion

Quality at harvest

At harvest, quality and sensorial characteristics of lamb's lettuce didn't significantly differ among the production systems with the exceptions of DHA and TPC-AA, as reported in Tab.1. While for TPC no significant differences could be observed among the systems, when phenols values were corrected for the ascorbic acid, SB samples showed higher content than the other systems. Lamb's lettuce were harvested at the same phenological state for the different systems, in fact, color, firmness, TSS, TA, Trolox and Vit-C were not affected by the production system confirming a similar maturity level.

Table 1. Organic lamb's lettuce quality attributes at harvest grouped by system of production.

Parameters \ Systems	AC	AM	SB	Anova P-values
L*	48.4	44.9	46.7	0.2650
a*	-16.8	-15.8	-16.4	0.1692
b*	21.1	18.7	19.5	0.3559
Chroma	27.0	24.5	25.5	0.2999
Hue°	-51.3	-49.9	-49.8	0.5385
Firmness (N)	56.1	57.8	55.6	0.8743
pH	6.093	6.075	6.102	0.8751
TSS (%)	6.1	6.7	6.5	0.4088
TA (%)	1.335	1.345	1.419	0.7537
Trolox (mg 100 g FW)	428.7	443.3	408.2	0.7859
AA (mg 100 g FW)	51.7	58.9	58.0	0.6424
DHA (mg 100 g FW)	**7.6 b**	**6.7 b**	**9.9 a**	**0.0018**
Vit-C (mg 100 g FW)	59.4	65.6	67.9	0.5762
TPC (mg gallic 100 g FW)	253.0	292.5	331.0	0.3075
TPC-AA (mg gallic 100 g FW)	**123.9 b**	**128.9 b**	**181.3 a**	**0.0270**

Same letter indicates no significant differences among the systems (Tukey, 0.05)

A possible explanation of phenols (TPC-AA) accumulation in SB is that this might indicate a plant stress adaptive response to nutrient deficiency. Before lamb's lettuce cultivation, soil mineral nitrogen was measured in each system as affected by previous crop uptake. SB resulted in the highest N availability and for this reason AM and AC received much more organic fertilizer than SB in order to compensate the initial unbalanced situation. During germination period, high amount of water is required but actually there is any root developed to uptake nutrients. Under this conditions the available nitrogen that is water soluble may be leached. SB, which had more mineral than organic nitrogen, was more exposed to leaching than AC and AM, resulting in moderate N deficiency. The relationship among nutritional stress and stimulation of phenolic metabolism is well documented in the literature (Lattanzio et al., 2009). Low nitrogen fertilization was found to be correlated with enhanced phenylalanine ammonia lyase (PAL) activity which stimulate the production of carbon based secondary metabolites (Ibrahim et al., 2011). In fact, PAL is the major enzyme of the phenylpropanoid pathway catalyzing the deamination of phenyalanine to obtain cinnamic acid (Dixon and Pavia, 1994). The removed ammine is a substrate of glutamine synthetase while the cinnamic acid is the precursor of the phenylpropanoids enabling the biosynthesis of stress-induced phenolic compounds. PAL activity regulation is known to be the branch point reaction between primary and secondary metabolism. (Cheynier, 2013).

A recent study confirmed that low nitrogen availability enhances secondary metabolism in organically produced tomatoes (Oliveira et al. 2013), which had both higher total phenols content and higher PAL activity than the conventionally ones. Shin and Schachtman (2004) observed that Arabidopsis roots responded to nutrient deficiencies by increasing the concentration of ROS that,, whichhave been suggested to mediate the sensing system for micronutrients deprivation. Definitively, ROS increase is involved in the oxidative stress and plant adaptation response (Mittler et al, 2004; Suzuki et al., 2012).

Moreover, in our experiment, SB lamb's lettuce showed the highest DHA content and accumulation has been frequently implied as biochemical indicator of oxidative stress in plant metabolism (Wise, 1995). In spinach leaves exposed to ozone fumigation DHA increased while AA remained constant during the stress (Luwe at al., 1993). In fact, the plant adaptation to adverse conditions leads to increase the ascorbate peroxidase (APO) activity (Foyer et al., 1995) which contributes to remove hydrogen peroxide, in case of ROS accumulation, by oxidizing ascorbate which is oxidized to DHA (De Tullio et al., 2013; Loewus and Loewus, 1987). Usually, the reduced form (AA) over the total vitamin C content is 90% (Pallanca and Smirnof, 2000); although, in lamb's lettuce, the initial AA concentrations were not affected by the production system, in SB leaves the lowest percentage of the AA/Vit-c (85%) was observed.

Quality during storage in MAP

In Table 2 are reported the results of the 3ways-ANOVA analysis (system x treatment x time of storage) of data during storage (Tab.2). Time of storage affected all the monitored attributes with the exception of L*, a*, Chroma and TSS%. Firmness, trolox, titratable acidity, ascorbic acid and phenols showed a similar trend characterized by a reduction over time; the production system only affected a*, TPC-AA and appearance score, while the packaging affected many parameters.

The tissue respiration combined with the gases permeability of the packaging film affected oxygen and carbon dioxide partial pressure in MAP headspaces during 11 days of refrigerated storage (Fig. 1).

Table 2. Significance of ANOVA for single factor, double and full interactions.

Parameter	Time	SYSTEM	Package	Time* SYSTEM	Time* Package	SYSTEM* Package	Time* SYSTEM* Package
L*	ns	ns	ns	ns	ns	ns	ns
a*	ns	***	*	***	*	ns	ns
b*	*	ns	*	ns	ns	ns	ns
Chroma	ns	ns	*	ns	ns	ns	ns
Hue°	***	ns	ns	*	ns	ns	ns
Firmness	***	ns	ns	ns	ns	ns	ns
pH	*	ns	***	ns	***	ns	ns
TSS	ns	ns	ns	ns	ns	ns	ns
TA	***	ns	***	ns	***	ns	ns
Trolox	***	ns	***	ns	***	ns	ns
AA	***	ns	ns	ns	ns	ns	ns
DHAA	**	ns	***	ns	***	ns	ns
Vit-C	***	ns	ns	ns	ns	ns	ns
TPC	***	ns	***	ns	*	ns	ns
TPC-AA	ns	***	***	*	ns	ns	ns
WL	***	ns	*	ns	ns	ns	ns
Odor	***	ns	***	*	***	ns	*
Appearance	***	***	***	***	***	*	***

Anova p values: * <0.05; ** <0.01; *** <0.001

After 1 day, oxygen rapidly decreased and simultaneously carbon dioxide increased. Significantly, at 4 days AM has accumulated more carbon dioxide, and has consumed more oxygen then AC and SB, which, under MAP conditions, have decreased their respiratory activity more that AM. The opposite situation resulted at 11 days; CO_2 increased at about 16%. and O_2 decreased close to zero for AC and SB that were significantly different from AM (10% of CO_2 and 6% of O_2). This indicates that the leaves response to MAP conditions established in AM resulted in a better control of the respiratory activity in respect to SB and AC leaves, at 11 days of refrigerated storage.

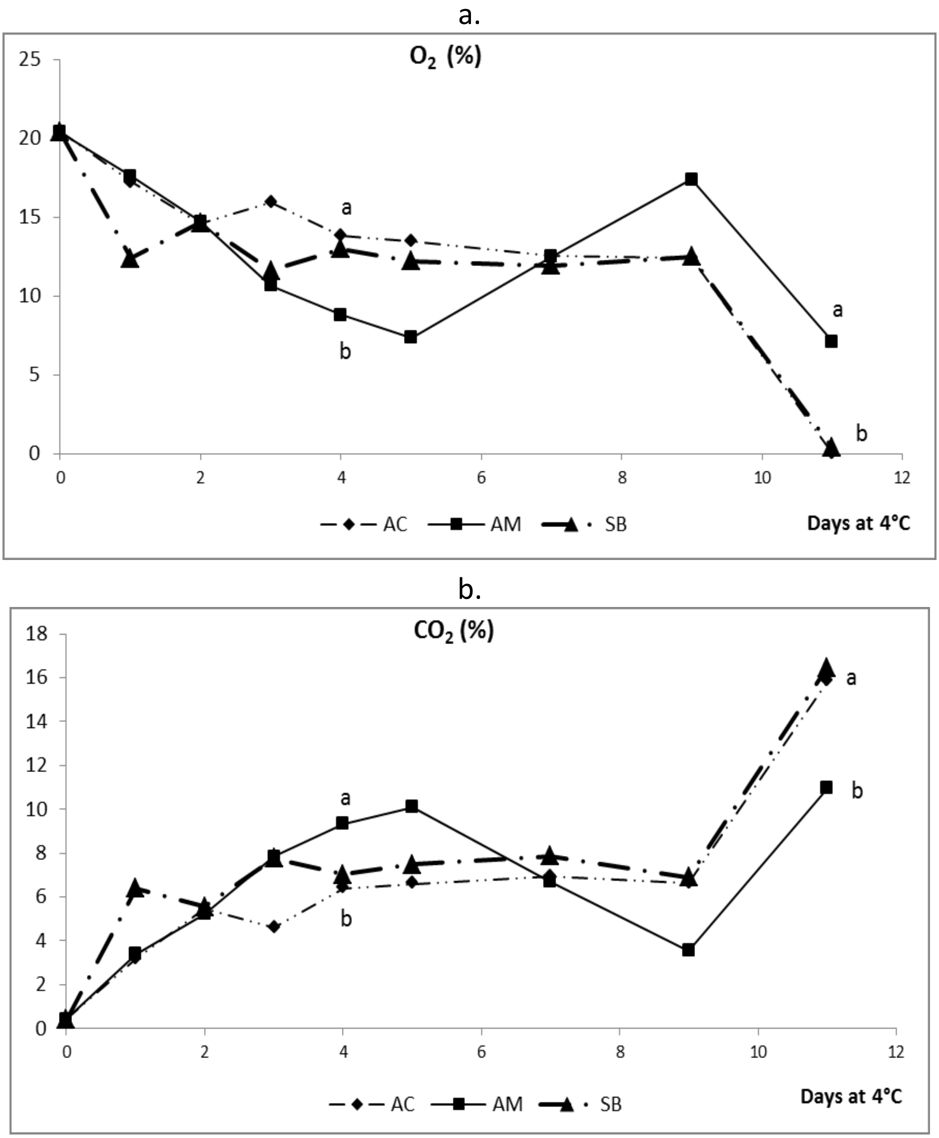
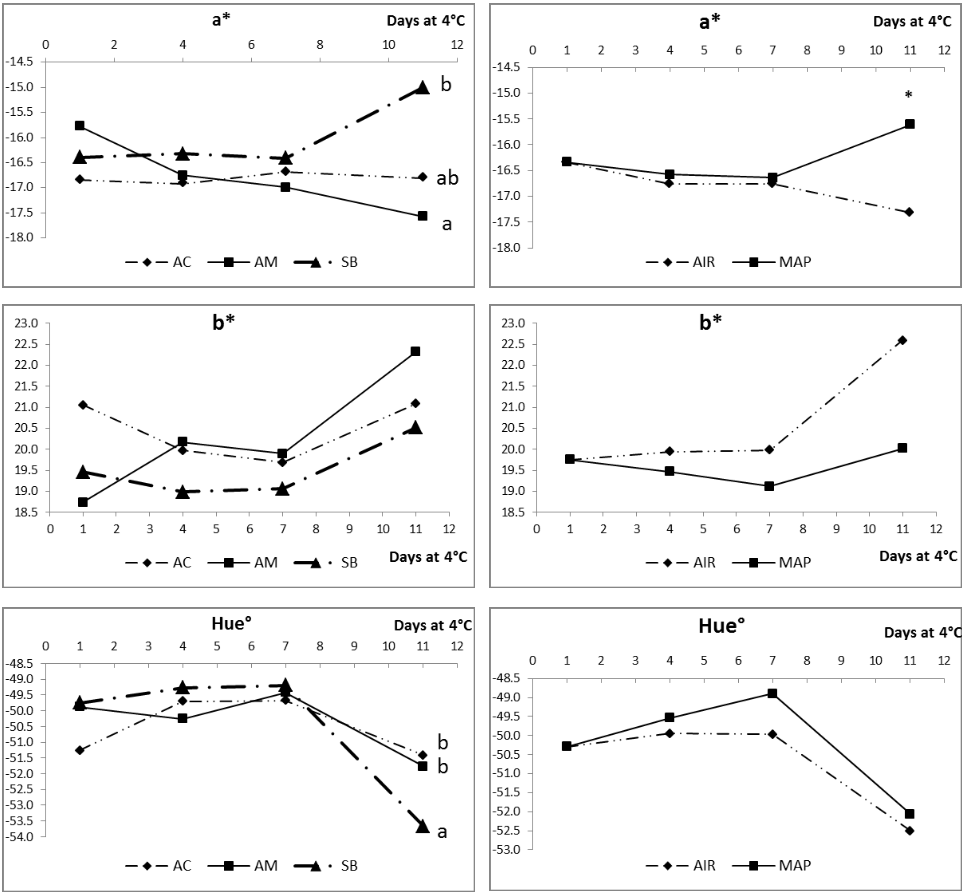


Figure 1. Oxygen (a) and carbon dioxide (b) evolution during storage in sealed bags (MAP) of lamb's lettuce produced from different fertility management systems. Atmospheric gas concentration is reported as initial values. Per each time of storage, different letters indicate significant difference (Tukey 0.05)

The storage conditions have affected the internal quality of the lamb's lettuce as reported in the ANOVA results (Tab.2). The full interaction of time, system and package affected only on the sensorial parameters, whereas no interaction system X package was observed, except that for appearance score.

For this reason, per each system the means values were averaged throughout the two package treatments to investigate the effect of the system on quality attributes over time. Similarly, per each package treatment the means averaged overall the three systems were considered. In Figure 2a is shown as an increase of b* was observed over time, indicating a yellowing process in the leaf. As for a* value a slight decrease was observed for AM while a clear increase was observed for SB samples, but always remaining in the negative part of the axes. At the end of the storage, hue angle variation showed higher color changes for SB samples than for the other systems, but with values moving versus more negative region. This indicates that SB leaves incurred in a more pronounced yellowing process compared to the other systems. As for the effect of the package, the differences were more evident at the end of the storage when a* value slightly decreased for AIR while highly increased for MAP samples. (Fig.3b). Similar behavior was observed for Chroma values (data not reported) that, at 11 days, were significantly higher in AIR then in MAP, on the other side, remained constant. As for b* and hue angle no significant difference were observed. These changes describe that MAP storage elicited leaves darkening over time, with leaves with leaves turning to olive-brown green at 11 days. As for sensorial perception of difference among systems and packaging conditions, appearance, to which color is one of the most relevant feature, confirmed that SB samples showed higher deterioration. After 7 days in AIR AM and AC systems reported higher scores than SB, and after 11 days appearance score resulted significantly higher in AC than in SB. Appearance in MAP at the end of the storage was higher for AM samples than t for the other 2 production systems.

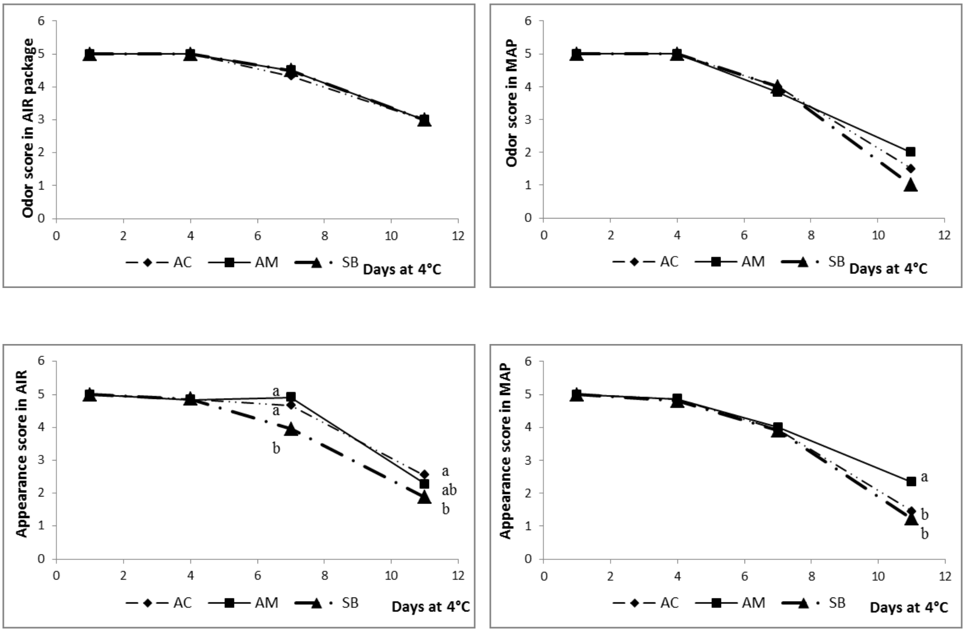


a. System by days of storage. Per each time, different letters indicate sign. diff. (Tukey 0.05)

b. Package by days of storage. Per each time of storage * indicates sign. diff. (p value<0.05)

Figure 2. – Changes of color parameters lamb's lettuce during storage

The effect of packaging was more evident on odor, than on appearance. At the end of storage, odor scores resulted significantly higher in AIR than in MAP (data not shown), where especially AC and SB recorded scores lower than AM reaching at 11 days score below 2. This was probably due to the low O_2 level achieved in MAP samples and in particular for SB and AC systems (Fig.1a). In fact, the oxygen percentage below the fermentation threshold induces anaerobic respiration leading to the production of off-flavours (Oms-Oliu et al., 2009).



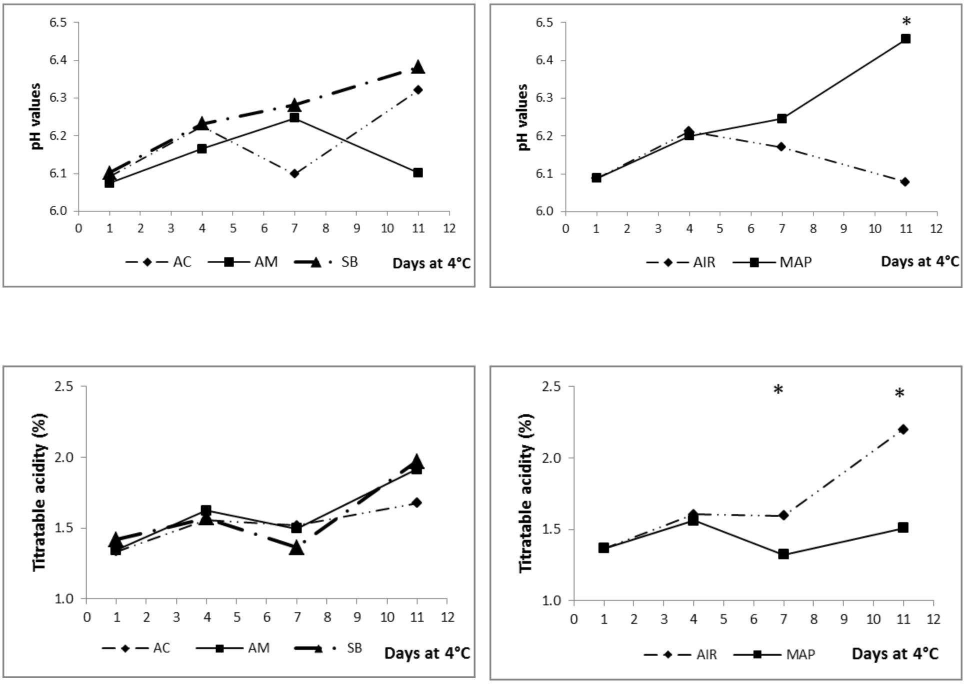
a. System by days of storage b. Package by days of storage

Fig. 3 – Sensorial scores of lamb's lettuce by production system grouped for MAP (a) or AIR (b) package. Per each time of storage, different letters indicate significant difference (Tukey 0.05)

It was expected, that the reduction of the respiration rate in MAP conditions could have contributed in preserving the appearance, maintaining nutritional quality, slowing the browning process and the rate of deterioration, well-documented in literature (Kader et al., 1989). However, for some commodities, high levels of CO_2 in the package might be not tolerated for a long period by plant tissues inducing the development of physiological damages and resulting in necrotic process as reported for basil (Amodio et al. 2005) and for artichokes (la Zazzera et al., 2012).

Although the tolerable CO_2 threshold for lamb's lettuce has not been investigated in specific studies, possible damages over 2% of carbon dioxide are reported for lettuce species and mixed salads, which also included lamb's lettuce (Saltveit, 1997). It is reported in literature that these products may well-tolerate very low oxygen content, close to zero (Beaudry, 2000), but no high CO_2. In the present experiment, soon after the first day of MAP storage the lamb's lettuce leaves were overexposed to carbon dioxide. The choice of the film and packaging conditions was made based on commercial information which suggest the use of PP and passive atmosphere. According to this, it might be that the excess of carbon dioxide accumulation isn't related to the film permeability, but it was due to the product versus headspace ratio which probably was too high. Actually, the lamb's lettuces in MAP were exposed to carbon dioxide much over the 2% which is suggested as tolerated threshold (Saltveit, 1997). What could have been between 3% and 5% wasn't investigated in this experiment and the minor differences reported between AIR and MAP invite to further test other MAP conditions. Furthermore, the role of oxygen shall be evaluated to assess the minimum tolerated pressure for lamb's lettuce in MAP conditions due to the possible fermentation processes which may take place in anoxic atmospheres. In fact, this might be linked to the odor scores decrease much reported in MAP than in AIR (Fig.3b).

As for chemical parameters, in Figure 4 are shown pH and TA changes over time. We can observe an increase of acidity and a corresponding decrease of pH in samples stored in air compared to MAP stored samples (Fig.4b), with statistical differences at 11 days for pH and at 7 and 11 days for TA.



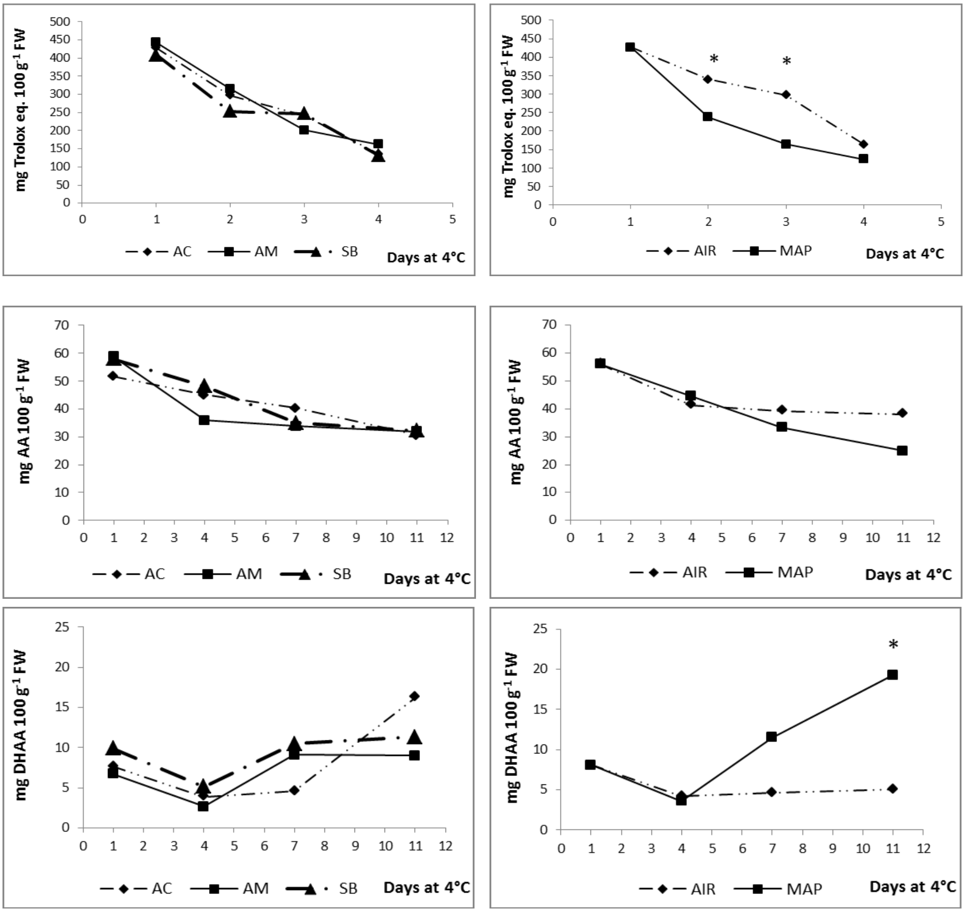
a. System by days of storage. Per each time of storage, different letters indicate sign. diff. (Tukey 0.05)

b. Package by days of storage. Per each time of storage * indicates sign. diff. (Anova, p value<0.05)

Figure 4. – Changes of pH and Titratable acidity of lamb's lettuce during storage

During the storage period no effect of the production system was observed for antioxidant composition (Fig.5a), whereas some difference could be attributed to the package atmosphere.

The initial differences recorded in the phenolic contents were lost during postharvest since 4 days of storage (Fig.6a). It means that after the harvest, lamb's lettuce leaves went through metabolic deteriorations which overtake the internal quality modification caused by the production system.

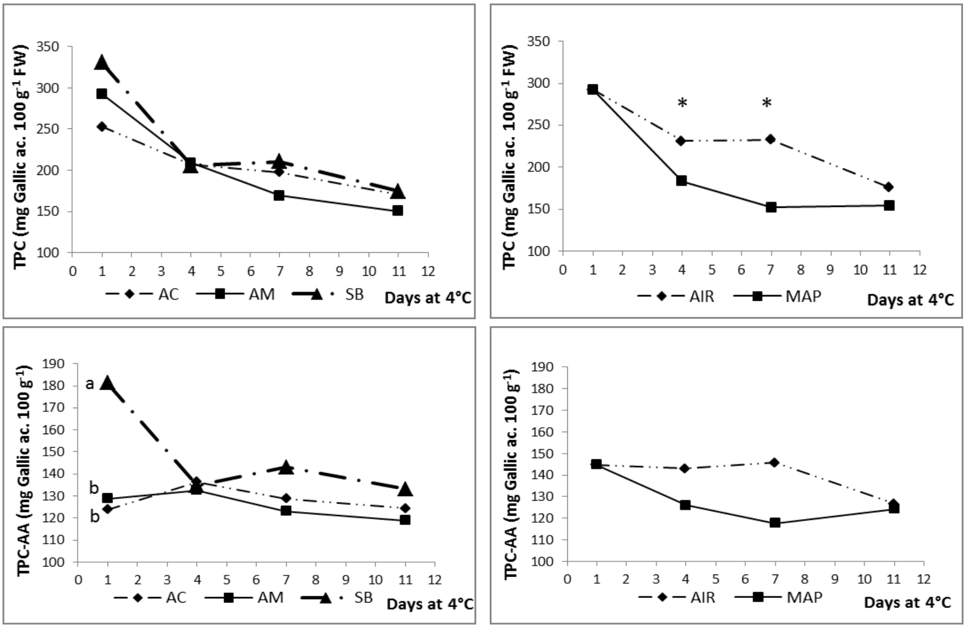


a. System by days of storage - Per each time of storage, different letters indicate sign. diff. (Tukey 0.05)

b. Package by days of storage. Per each time of storage * indicates sign. diff. (p<0.05)

Figure 5. – Changes in antioxidants and Vitamin C components of packaged lamb's lettuce during storage

TPC concentrations decreased during storage in contrast with the results reported in literature for the same crop (Ferrante, 2009). However, the TPC-AA values remained constant in time. The TPC-AA values resulted quite lower than the TPC indicating a relevant interaction of AA molecules during the TPC determination assay. In fact, AA showed a decreasing trend during storage. This is probably due to the oxidation in DHA that, instead, has increased in time. These results are consistent with those previously reported by Gil et al. (1999) on fresh cut spinach stored at 10°C both in air and modified atmosphere.



a. System by days of storage - Per each time of storage, different letters indicate sign. diff. (Tukey 0.05)

b. Package by days of storage. Per each time of storage * indicates sign. diff. ($p<0.05$)

Figure 6. – Changes in phenols of packaged lamb’s lettuce during storage

As far as the package effect is concerned, MAP resulted in significantly lower level of antioxidant activity and TPC than AIR, at 4 and 7 days of storage; at 11 days also DHA in MAP resulted higher than in AIR (Fig.5b). This is a consequence of the modified gas composition. In fact, it was already reported the olive-browning effect of MAP at 11 days of storage that, according to Løkke et al. (2012) might be related with the loss of tissue integrity. This damages has affected the internal quality of lamb’s lettuce at the end of storage period, as resulted by the return of TPC and trolox in MAP in respect to AIR and by the depletion of AA and, in consequence, the simultaneous increase in DHAA that leaded to significantly higher DHA accumulation in MAP then in AIR (Fig.5b).

6.4 Conclusion

The production system contributes in defining the quality characteristics of fresh fruits and vegetables at harvest but a limited amount of researches investigated its impact on quality during postharvest storage. This research focused on the effect of different organic soil fertility management strategies and modified atmosphere packaging on quality and shelf-life of lamb’s lettuce leaves. At harvest, an accumulation of phenols and dehydroascorbic acid in the system with the lowest initial supply of organic amendment (namely SB) was observed. A moderate stress for nutrient starvation has been suggested as possible explanation for this effect. In fact, the agroecological systems, preserved the highest rate of ascorbate pool in reduced form as a result of a well-adapted organic fertilization. This result showed also that high input of organic amendment supply, as it was applied in AM and AC, is a feasible practice to satisfy the nutritional requirement of the lamb’s lettuce, a short cycle crop, while in general, organic matter based amendments are used to provide available nutrients in limited amount but for a long period of time.

During storage, air package succeed to maintain the initial quality attributes for a longer period in comparison to the modified atmosphere package that, at 11 day despite the production system, developed off-odors under the threshold of acceptability. This result suggested that carbon dioxide accumulation and oxygen depletion should be avoided.

In conclusion, the initial quality of organic lamb's lettuce fertilized with high input of organic amendment was best maintained during air storage at 4°C. Further researches may focused on a better understanding of the mechanism that connect nutrient deficiency and stress response in organic lamb's lettuce, investigating a wide range of nutrients and of enzymes involved in the secondary metabolites pathways.

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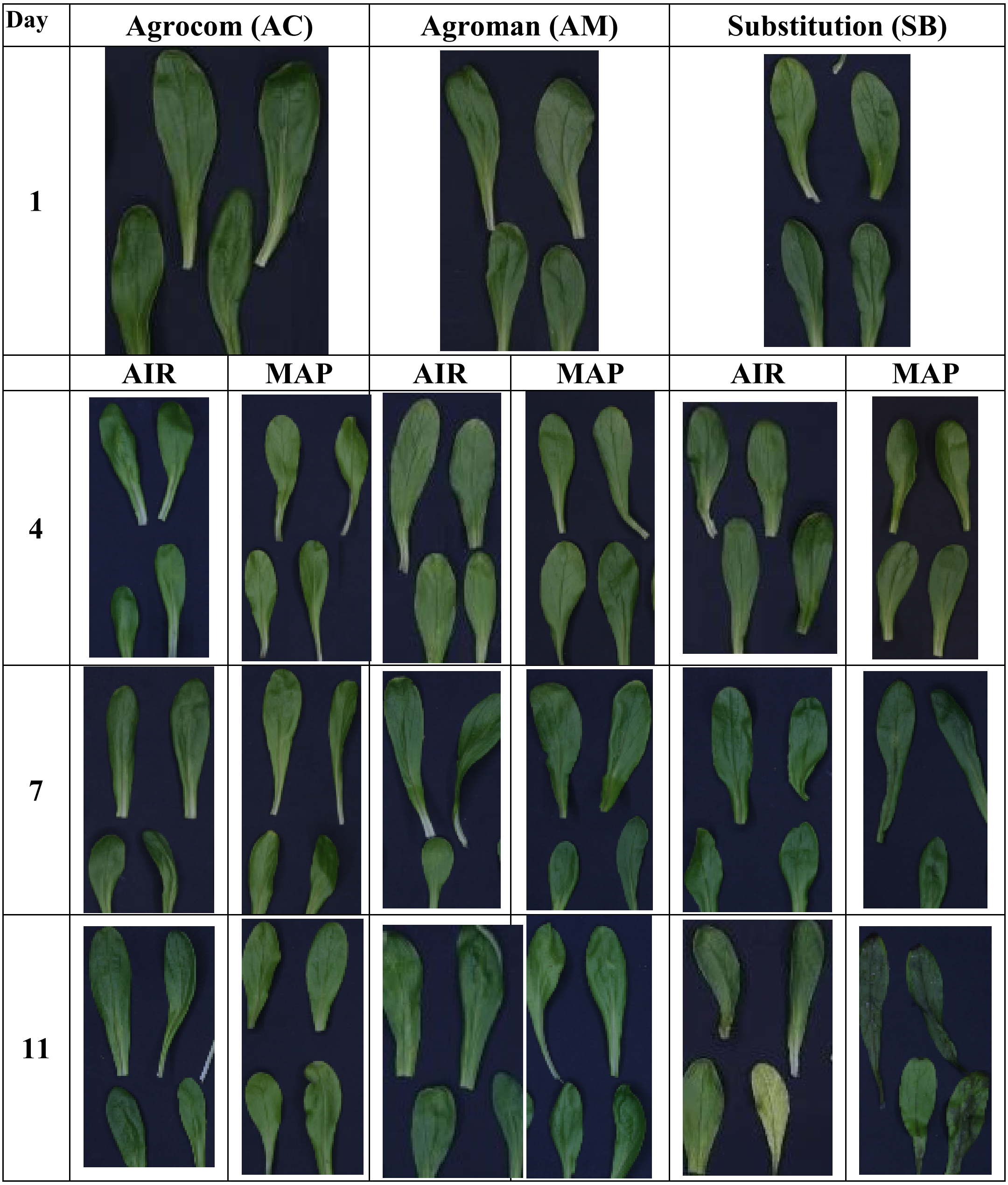
Picture of lamb's lettuce in the packages



Pictures of lamb's lettuce leaves at each sampling time

7. Postharvest Evolution Of Quality And Nutritional Attributes Of Organically And Conventionally Grown Strawberries

Abstract

Health benefits, nutritional values, safety and absence of pesticide residues have been identified, much more than environmental concern, as the primary reasons for purchasing organic food. However, the scientific basis of the differences between organically and conventionally produced foods are still under debate. The aim of this research was to compare the quality characteristics and the postharvest performance of 'Festival' strawberries organically and conventionally grown. Three organic farming systems, differentiated by fertility management, were implemented in the experimental greenhouse at the Mediterranean Agronomic Institute of Bari and were compared to one conventional system in a nearby commercial greenhouse with similar soil characteristics and facility. The four systems are described as follows: a conventional farming system based on mineral fertilizers (CONV); a simplified organic production system based on organic commercial fertilizers (SUBST); two organic production systems based on agro-ecological practices, AGROMAN, amended by using animal manure and cover crops incorporation and AGROCOM, amended by on-farm compost and green manure. After harvest, fruits were stored at 0°C under a continuous humidified air flow. At 0, 4, 8 and 14 days, the respiration rate, colour, firmness, acidity, soluble solids, pH, vitamin C, phenols, organic acids and sugars were monitored and sensorial attributes assessed. At

harvest, conventionally grown strawberry fruits resulted higher in diameter, and firmness compared to all the organically produced strawberries. Vitamin C content was higher in SUBST than in CONV; TSS, fructose and glucose contents were higher in AGROMAN then in CONV. During postharvest, initial differences in firmness, glucose and fructose and Vitamin C were maintained with also a significant accumulation of oxidized vitamin C in CONV. At the end of storage, total phenols were significantly lower in CONV than in AGROMAN and AGROCOM. In conclusion, the production system affected the quality parameters of 'Festival' strawberry cultivated in greenhouse under Mediterranean climate conditions. Organically grown strawberries resulted in higher nutritional compounds at harvest and maintained their initial quality better than conventional strawberries. A principal component analysis resulted in complete separation of conventionally grown strawberries from the other, but also it was able to identify relevant differences in the quality and postharvest attributes among the three organic systems. Possible explanations for the effects of production system on nutritional quality and postharvest performance of fresh produce are considered the diverse mineralization of fertilizer and amendment supplied and the secondary metabolites accumulation as plant stress response.

7.1 Introduction

Strawberries belong to Rosaceae botanical family and they produce an aggregate fruit (in general considered as strawberry fruit) with the outside part composed by the achenes (each one holds a seed and it is a true fruit derived from flower ovaries) and the fleshy part with accessory material that is derived from the flowers receptacle. Strawberry is a non-climacteric fruit that doesn't respond to ethylene stimulation at any stage of the maturity process (Manson and Jarvis, 1970). For this reason, it's critical to harvest the fruits at the optimum stage of ripening in order to achieve maximum initial quality (Sturm et al., 2003). Early picking does not guarantee high organoleptic quality in terms of taste, color and texture, whereas late harvest may lead to soft berries, and thus more sensitive to physical damage and to microbial contamination during handling and postharvest operations (Kader, 1991). In fact, the infection of *Botrytis cinerea* is a major fungal disease reducing the storability of strawberry fruits (Sanz et al., 2002). Being a fruit so perishable, postharvest storage conditions have been deeply investigated to prolong the shelf life of this fruits. Strawberries have high respiration rate, about 10-15 mg CO^2 kg^{-1} h^{-1} at 0°C, that increases 4-5 times at 10°C (Kader 1991), suggesting to keep storage temperature as close to 0°C as possible. Modified atmosphere condition reduces the respiration rate contributing to preserve the visual appearance, and the nutritional quality of strawberries (Kader et al., 1989; Kader 1991; Nunes and Morais, 2002; Al-Jamali and Hani 2006). Atmosphere with 0.5% of oxygen are reported to control fungal decay, but lower levels induces noticeable off-flavors. Also elevated CO_2 concentrations, over 30%, help to prevent fungal diseases but when the berries return in air they show residual effect on firmness, color and decay percentage. Optimal atmosphere should therefore to be limited to concentration range of 0.5-5% O_2 and 10-20% CO_2. Controlled atmosphere remains a valid complementary tool to the use of cold temperature; above 0°C, controlled atmosphere (5%O_2+15%CO_2)

resulted more effective at 4°C than 10°C in maintaining strawberries quality for two weeks (Nunes and Morais, 2002). Other strategies are under investigation to reduce the respiratory activity and to maintain the initial quality of strawberries during storage. Numerous postharvest treatments, as edible coating, hot air or water, aqueous chloride dioxide, putrescine, ascorbic acid, calcium salts, have been evaluated to sanitize the fruits and prevent postharvest diseases, (Asrey and Jain 2005; Lara, 2006; Khosroshahi et al., 2007; Amal et al., 2010; Aday et al. 2013) but with contradictory results according to variety and ripeness of the berries.

Although this large amount of studies on postharvest storage of strawberries very few are focused on organically managed fruits and on the effect of the growing system on the postharvest performance. Organoleptic characteristics, and resistance to deterioration resulted higher for organic strawberry in comparison to conventional fruit during simulated marketing conditions (Cayuela et al. 1997). Organic strawberries with higher anthocyanin contents than the conventionally grown were found after storage at 0°C (90-95% RH) in AIR (Abu-Zahra et al., 2007) while MAP stored fruits resulted with no significant differences. Nunes and Delgado (2014) compared organic and conventionally grown strawberries purchased at the retail in Florida and California, and reported that organic grown fruits tended to have higher acidity, total phenols and ascorbic acid contents than conventional fruits, whereas sensory quality and texture resulted similar in all the systems.

Studies on other fruit species, reported differences during conservation in terms of incidence of physiological disorders, postharvest diseases, physical and internal quality and mineral as on apples (DeEll and Prange, 1993), banana (Nyanjage et al., 2001), lemons (Uckoo er al., 2015), and kiwifruits (Hasey *et al.* 1997; Benge *et al.* 2000; Amodio at al., 2016).

To this regard, the objective of the present research is to compare the quality at harvest and during postharvest storage of strawberry 'festival' fruits produced with different organic approaches (inputs) in comparison to conventional fruits grown in similar pedoclimatic conditions.

Organic production system is made of a series of very diverse approaches that relies on different strategies to implement the crop sequence in rotation, to decide sources, quality, timing and doses of water and fertilizers, to include cover crop, to manage weed and to control pathogen and disease. Diverse farming systems might lead to different product quality that during storage might turn into different postharvest evolution and shelf-life. As a general consideration, soil fertility management in organic production should be based on crop rotation, including cover crops and legumes, with a particular attention in crop species sequence and appropriate variety selection. With very few exceptions, external nutrient input shall be provided by organic amendment and fertilizers. The plant needs during the whole crop cycles are covered by the nutrients mineralization of exogenous organic biomasses and soil organic matter pool which depend on a series of factors including pedoclimatic variables, amount, time and system application for the input, origin and stability of the organic raw materials in the fertilizers and soil microbial biomass and activity which are the most important ones (Ceglie and Adbelrahman, 2014). When this strategy is not enough to satisfy plant demand of nutrients, the recovery actions are very limited. The only alternative is to apply organic fertilizers in liquid forms that provide much more soluble nutrient than the organic amendment and solid state fertilizers but can't be compared with the mineral fertilizers, allowed only in conventional farming, that are easily and almost completely soluble. It results that plants organically grown, especially the short-time cycle crops, have to face different abiotic stress conditions either for the pathogen attacks, either for nutrient starvation (Oliveira et al.; 2013). The latter, may be of strong or moderate intensity, short or extended duration, for single or multi nutrients, according the aforementioned factors which affect the nutrient availability and uptake and also according the specific fertility management strategy.

For all these reasons is of relevant importance to study the effect of organic management and eventual abiotic stresses on quality and storability of organically in comparison to conventional grown fruits.

7.2 Materials and methods

Experimental design

Three organic farming systems, differentiated by fertility management, were implemented at the experimental greenhouse facility of the Mediterranean Agronomic Institute of Bari (Apulia region, Italy, 41°.0553 N; 16°.8754 E) and were compared to one conventional system monitored in a nearby commercial greenhouse (Valenzano – BA – 41°.02981 N; 16°.8749 E) with similar characteristics of soil and tunnel structure and coverage material.

The farming systems under comparison were: i) SUBST, consisting in an organic input substitution widely adopted in organic production system, especially in greenhouse horticulture, which mimics conventional agriculture by substituting conventional agrochemicals with allowable organic products; ii) AGROMAN, characterized by the use of a cover crop mixture (based on legume species) and mature manure (from organic husbandry); and iii) AGROCOM, which uses a different cover crop mixture (mainly based on brassica species) and compost produced on-farm (at the experimental composting facility of MAIB) and iv) CONV, a conventional farming system based on 100% mineral fertilizers. The three organic farming systems were randomly assigned in three replications for a total of 9 plots (3.0 x 4.0 m) in a complete randomized block design. Additional information about the crop rotation in witch this experiment has been included are reported in previous work (Mihreteab et al., 2014).

Cultural practices and plant material

‘Festival’ strawberry roots, certified as organic from the same nursery (f.lli Zanzi, pd Italy) were used both for organic and conventional productions. In all the systems, they were transplanted on late September 2012 managing in this way the begin of the productive stage for March 2013. In April, all ripen fruits were hand harvested from three different plots of the same systems for a total of 12 experimental units.

Samples were quickly delivered to the postharvest laboratory at the University of Foggia and divided into 4 subsets of about 500 g for each replicate to monitor the storage performances at 0, 4, 8 and 14 days after harvesting. Fruits were stored at 0°C in plastic jars flushed with a continuous humidified air flow (set according to the respiration rate in order to not accumulate more than 0.2% CO_2). During storage at 0, 4, 8 and 14 days the respiration rate, physical and chemical quality characteristics and sensorial attributes were monitored.

Respiration rate

Fruit respiration rate (mL CO_2 kg^{-1} h^{-1}) was calculated using the dynamic system (Kader, 2002). Carbon dioxide concentration was measured using a gas chromatograph (Shimadzu 17A) equipped with a TCD detector (200 °C) then corrected for the weight of the sample, and for the air flow as measured by a flow meter (ADM100 Agilent Technologies, Inc. 2850 Centerville Road Wilmington, US).

Physical quality characteristics

Fruit diameter was measured with a digital caliper. Color was measured on two sides of the fruit, using a colorimeter (CM-2600d, , Minolta, Osaka, Japan) in the CIE L*a*b* mode, then $\text{Hue}° = \tan^{-1}\frac{b*}{a*}$ and $\text{Chroma} = \sqrt{a*^2 + b*^2}$ were calculated from L*, a* and b* values.

Firmness was determined on 10 strawberry fruits for each replicate as percentage of fruit deformation under 2 parallel plates with a force applied of 5N (kg m s^{-2}) by using an Universal Testing Machine (INSTRON 3343 Norwood, MA, US).

Chemical quality characteristics

Each fruit was squeezed and the obtained juice drops were used for direct readings of total soluble solids (TSS%) content with a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan).

Total acidity (TA) and pH were measured on 5 g of squeezed juice for each replicate, using an automatic titrator (TitroMatic CRISON, Spain) with 0.1 mol L^{-1} NaOH solution up to final pH of 8.1 and reported as percentage of citric acid per 100 g.

Organic acids and sugars were extracted homogenizing 5 g of fresh strawberry tissue with 10 ml of ultrapure water using IKA T18 Ultraturrax (Wilmington, USA) homogenizer at 14000 rpm for 1 min. The homogenate was centrifuged at 9000 rpm for 10 minutes at 5°C. The supernatant was filtered with a C_{18} Sep-Pak cartridge (Grace Pure ™ , New York, USA) and then with a 0.2 µm filter (INCOFAR, Modena, Italy).

Organic acids and sugars were identified using the method described by Mena et al. (2011).

Vitamin C (Vit-C), L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), content was determined as described by Zapata and Dufour (1992) with some modifications (Gil et al., 1999). Total phenols were determined according to the method of Singleton and Rossi (1965).

The content of total phenols was calculated on the basis of gallic acid calibration curve and expressed as mg of gallic acid per 100 g of fresh weight of strawberry fruits.

Sensorial attributes

A blinded panel test was performed by a group of six trained panelists to assess fruits appearance, color, acidity, aroma, sweetness on a qualitative 5 to 1 scale (5= excellent, 3=fair, limit of marketability, and 1=very poor).

Off-flavor were evaluated from 1 to 5 where 1 denoted typical flavor and 5 denoted very intense off flavors.

Statistical analysis

The significant level of production systems, time of conservation and their interaction were evaluated on all the measured parameters by two ways ANOVA (STATISTICA 8.0, Statsoft inc., Tulsa, OK, US) e post-hoc Tukey ($p \leq 0.05$).

The overall variability of the recorded dataset were investigated by principal component analysis in order to study the multivariate effects on the systems characteristic at harvest and at the end of storage.

7.3 Results and discussion

Quality at harvest

Physical and chemical characteristics at harvest

Significant differences of physical attributes at harvest concerned the diameter, and firmness (Tab. 1). CONV strawberries had highest diameter, while no differences were observed among the other systems. The initial quality didn't present differences in color parameters suggesting that samples were harvested at the similar level of maturity in any production system under investigation. In fact, widely used strawberries ripeness index is based on the percentage of the fruit skin area of pink-red colored (Mitchell et al., 1994).

As for firmness, AGROCOM strawberries were much softer than CONV, showing an higher relative deformation; intermediate values were observed for the other two organic systems. It is possible that the distension of cell walls, due to water intake, leaded to turgid fruits with increased diameter and firmness; consequently, a dilution effect might have affected the TSS content (Tab.2) which was lower in CONV than in SUBST and AGROCOM, with medium values for AGROMAN.

Table 1. – Effect of the production system on physical attributes at harvest of strawberry fruits,

Attributes	AGROMAN		AGROCOM		SUBST		CONV	
D (mm)	30.4	b	28.2	b	28.8	b	37.9	a
L*	38.1		40.1		39.8		36.0	
a*	22.1		21.8		21.8		26.0	
b*	4.5		4.2		6.3		5.0	
Chroma	22.7		22.3		23.0		26.6	
Hue°	11.1		10.4		15.8		10.7	
Firmness (def%)	9.3	ab	11.6	a	9.3	ab	7.3	b

For each parameter, different letter indicates significant differences among means (Tukey, P<0.05)

In present study, organic acids at harvest were not affected by the system of production (Tab.2), while, sugars content and composition showed significant differences that might be affected the resulting TSS percentage. In fact, CONV and AGROMAN fruits had lower Glucose and Fructose content than SUBST, with AGROCOM in-between, while no differences were reported in the Sucrose content.

Table 2. – Effect of the production system on chemical attributes at harvest of strawberry fruits.

Attributes	AGROMAN		AGROCOM		SUBST		CONV	
TSS (%)	9.5	ab	10.6	a	11.2	a	7.0	b
pH	3.863		3.980		3.883		3.823	
TA (mg citric ac. 100 g^{-1})	0.773	a	0.658	ab	0.597	b	0.580	b
Citric ac (mg 100 g^{-1})	366.4		358.1		374.7		425.7	
Malic ac. (mg 100 g^{-1})	384.9		434.9		460.1		316.8	
Succinic ac. (mg 100 g^{-1})	51.5		40.4		59.9		58.1	
Fumaric ac. (mg 100 g^{-1})	0.7		1.1		1.5		1.3	
Tartaric ac. (mg 100 g^{-1})	29.8		36.0		38.1		33.8	
Glucose (mg 100 g^{-1})	2062.9	b	2556.6	ab	2979.6	a	1840.8	b
Sucrose (mg 100 g^{-1})	785.8		1035.0		653.5		689.9	
Fructose (mg 100 g^{-1})	274.5	b	346.5	ab	398.8	a	247.1	b
AA (mg 100 g^{-1})	29.3	a	22.6	b	19.3	b	11.9	c
DHA (mg 100 g^{-1})	3.0	a	2.8	ab	2.5	ab	1.1	b
Vit-C (mg 100 g^{-1})	32.3	a	25.4	b	21.8	c	13.0	d
TPC (mg gallic ac.100g^{-1})	95.3		109.8		129.2		102.4	

For each parameter, different letter indicates significant differences among means (Tukey, $P<0.05$)

Ascorbic acid and total vitamin C resulted significantly lower in CONV than in all the organic systems. In particular, ascorbic acid was higher in AGROMAN than in AGROCOM and SUBST and both had higher content than CONV; dehydroascorbic acid was higher in AGROMAN than CONV with intermediate values in AGROCOM and SUBST.

The sum of AA and DHA, both relevant for their nutritional role, resulted in four significantly different groups of vitamin C contents decreasing from AGROMAN, AGROCOM, SUBT and CONV. Furthermore, the percentage of reduced form of vitamin C pool in CONV (82%) resulted significantly lower (p=0.0005) than in the organic strawberries (average of 92%).

As sensorial attributes reported in Tab.3, at harvest appearance, color, aroma, acidity, sweetness and off-odors were not statistically affected, despite the relevant effects aforementioned of the systems of production system on the initial quality of the strawberry fruits.

Table 3. – Effect of the production system on sensorial attributes at harvest of strawberry fruits.

Attributes	AGROMAN	AGROCOM	SUBST	CONV
Appearance score	3.9	3.7	3.6	4.5
Color score	4.1	4.0	4.0	4.6
Acidity score	3.7	3.6	3.5	3.7
Sweetness score	2.4	2.8	2.4	3.0
Off-flavour score	1.0	1.0	1.0	1.2

For each parameter, different letter indicates significant differences among means (Tukey, P<0.05)

Furthermore, it shall be stressed that, since strawberries were cultivated in plastic tunnels, water regime, sunlight, and temperature were kept under control decreasing the fruit quality dependency fromthe environmental conditions (Sturm et al., 2003). For this reason, the differences recorded at harvest mainly depend on the implemented fertilization strategies which, at the end, release available nutrients in different quantity and moment regardless of optimal plant requirement.

During the whole crop cycle, nutrient starvation might occur leading to moderate plant stress which cause, directly or indirectly, accumulation of reactive oxygen compounds (ROS). ROS are also associated with sugar accumulation as plant adaptive response to the stress conditions, which may be the reason of the highest sugar content observed in organic strawberry fruits (Roitsch, 1999). In the study of Wang and Lin (2003) on strawberries 'Allstar' and 'Honeoye' the use of compost increased, among the other parameters, fruits AA, GSH, flavonol and the rate AA/DHA and GSH/GGSG eliciting the antioxidant properties of strawberries against ROS. Organic farming relies on the use of organic fertilizers and amendments which have much lower nutrients solubility than mineral fertilizers chemically synthetized. This means that the synchronization between plant demands and nutrients availability and, moreover, the right balance among several necessary nutrients are not easily ensured in organic farming. However, the nutrient starvation is possible also in case of conventional production; Anttonen et al. (2006) reported that for strawberry 'Bounty' under different dosage of mineral fertilizers, an increase of 57% for quercetin, 19% for kaempferol and 21% for ellagic acid in fruits grown at the lowest fertilization level. Abu-Zahra et al. (2006) have reported the lowest TSS in strawberries 'Honor' conventionally produced and an increasing percentage of TSS with the increase of organic matter content in the fertilizer applied for the organic strawberries production. The content of TSS is the function of several plant cell compounds of which total sugars and organic acids constitute the major part (Sturm et al., 2003). In this present study, organic acids at harvest did not change among the investigated system of production (Tab.2), while, individual sugar contents presented significant differences (Tab.3) that might have affected the resulting TSS percentage. CONV and AGROMAN showed, in fact, lower content of glucose and fructose than SUBST, with AGROCOM showing intermediate content, while no differences were reported in the sucrose content. In this respect, it is possible to speculate that high carbohydrate supply, due to low nitrogen availability at a different extent among the systems, resulted in the diversion of the metabolism from protein to secondary metabolites biosynthesis.

Similar hypothesis is reported in literature for organic tomatoes with higher TA, TSS, TPC and AA than the conventional tomatoes (Oliveira et al., 2013). Although the aforementioned evidences, the relationship between stress and sugar accumulation in fruits may be difficult to be interpreted because glucose is the main carbon precursor for the amino acid skeletons of the primary metabolism but also for carotenoids and ascorbate (Couée et al., 2006). Lee and Kader (2000) in their review reported many studies that described a decrease in vitamin C concentration in fruits and vegetables supplied at high rates of mineral nitrogen.

A possible hypothesis might be that glucose accumulated through the photosynthetic activity is utilized much more as substrate for the ascorbic acid biosynthesis than for the primary metabolism pathway resulting in ascorbic acid accumulation. Reganold et al. (2010) have related the quality of the strawberries ‘Diamante’, ‘Lanai’ and ‘San Juan’ with the soil quality at the production sites. In their results, organically managed soils had highest total nitrogen, total carbon and microbial activity and as consequence, fruits showed higher total antioxidant activity, ascorbic acid, and total phenols than conventional ones. In the present experiment, Vitamin C values at harvest resulted in four statistically distinguished groups with the decreasing concentrations for AGROMAN, AGROCOM, SUBST and CONV which someway is opposite related to the mineralization rate of the nutrient input supplied at each system: mineral fertilizer in CONV, organic fertilizers in SUBST and organic amendments, namely compost and manure in AGROCOM and AGROMAN respectively.

Clearly, the solubility of nutrients decrease from mineral to organic fertilizers and then to organic amendments, however it might be expected higher mineralization in manure amendment than in compost. It is confirmed that organic amendments, both compost and manure, elicit secondary metabolite and antioxidant biosynthesis pathway as already discussed, but in case of compost application there might be a mechanism, other than the nutrient based one, such as the hormone-like effect (Bernal-Vicente et al., 2008) that stimulate the plant growth, because compost contains lignin degradations catabolites that have auxin-like activity, results in a compensated equilibrium of primary-secondary metabolism.

Quality during storage

Production system and storage time significantly affected physical and nutritional quality attributes of strawberries either as single factor or as effect interaction, as resulted from the ANOVA (Tab.4).

Table 4. Significance of P-values according to ANOVA two-ways for production System, storage Time and their Interaction.

Physical quality attributes

	SYSTEM	TIME	SYSTEM x TIME
L*		***	*
a*			
b*	**	*	
Chroma			
Hue°	***	**	*
Firmness (def%)	***	*	

Chemical quality attributes

	SYSTEM	TIME	SYSTEM x TIME
TSS (%)	***		
pH			
TA (mg citric a. 100 g^{-1} FW)	*	**	***
AA (mg 100 g^{-1} FW)	***		*
DHA (mg 100 g^{-1} FW)		***	***
Vit-C (mg 100 g^{-1} FW)	***		***
TPC (mg gallic a. 100 g^{-1} FW)	**	***	**
Citric a (mg 100 g^{-1} FW)		***	
Malic a. (mg 100 g^{-1} FW)	***	*	
Succinic a. (mg 100 g^{-1} FW)	*	***	*
Fumaric a. (mg 100 g^{-1} FW)			
Tartaric a. (mg 100 g^{-1} FW)	*		
Glucose (mg 100 g^{-1} FW)	***		
Sucrose (mg 100 g^{-1} FW)		*	*
Fructose (mg 100 g^{-1} FW)	***	*	

Sensorial attributes

	SYSTEM	TIME	SYSTEM x TIME
Appearance score	*	***	
Color score	*	***	
Acidity score		**	
Sweetness score	***		
Off-flavour score	*	*	

* = P<0.05; **=P<0.01; ***=P<0.001

Respiration rate

In Fig. 1 is reported the respiration rate of the strawberries during storage. The differences in the initial values with AGROMAN presenting the highest respiration rate and CONV the lowest were not significant. For all the system a rapid decrease during the first four days was observed, followed by an increase (about 13 ml CO_2).

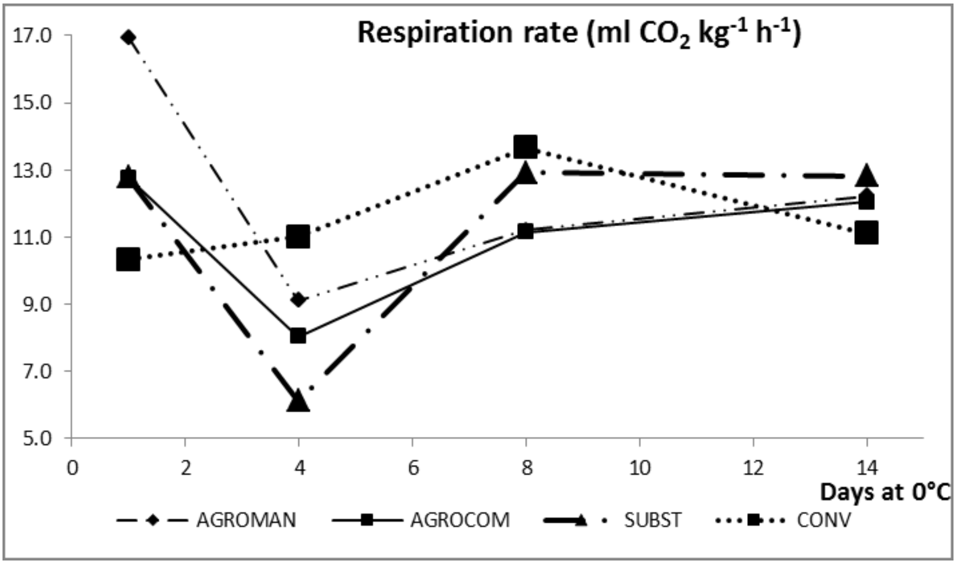


Figure 1. Effect of the production system on the respiration rate of strawberry fruits stored 14 days at 0°C.

Physical attributes

Strawberries color significant changes regarded L*, b* and Hue° (Tab.4). Chroma and a* remained constant and similar among the systems. L* slightly decreased over time indicating skin darkening which was evident only at 4 days when L* was lower in all the organic system than in CONV (data not shown). The changes of b* and Hue° presented similar behaviors and they appeared much evident at 4 days of storage with significantly lower values of conventional strawberries in respect to the organic ones (Fig. 2). In general, this color differences were very limited. It may be shortened that conventional strawberries were slightly more scarlet-red colored than the others that instead were a little towards red-orange color.

Firmness slightly decreased in time (Fig.2), as indicated by the increase of relative deformation, as consequence of fruits deterioration, with the exception of AGROCOM that had constant firmness trend, taking into account that its initial deformation values were already low. The differences recorded among the systems at harvest were maintained constant during the whole storage period.

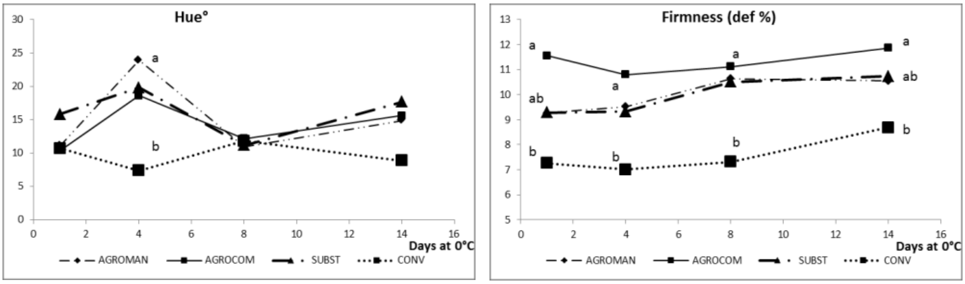


Figure 2. – Effect of the production system on Hue° and firmness of strawberry fruits stored 14 days at 0°C. Per each time, letter indicates homogeneous group of means (Tukey, $P<0.05$)

Chemical attributes

Strawberries pH values remained around initial values (Tab.1) for 8 days (4.3 AGROCOM; 4.2 SUBST; 4.1 CONV and 4.0 AGROMAN), then slightly increased in CONV (about 4.4) and it decreased in SUBST (3.7) with no statistical difference among treatments (data not shown).

Other studies on strawberry storage confirmed constant pH during the time, as also expected for non-climacteric fruits (Ayala-Zavala et al., 2004; Nunes et al., 2002).

Titratable acidity and organic acids concentration recorded during storage in the different systems are reported in Fig.3. In time, the initial differences in TA, whit AGROMAN showing higher values than AGROCOM and SUBST, disappeared due to a decreasing trend which was much more fast in AGROMAN than in fruits from the other systems. A similar decreasing trend was recorded for citric acid (not shown), with no significant differences, and in succinic acid profile, but with the exception of AGROMAN that remain almost constant. In SUBST fruits the initial succinic ac. decreased for the 80% and at the end of the storage resulted significantly lower than AGROMAN. Initial levels and differences in malic acid remain the same during storage. In general, CONV had lower malic ac. than the organic fruits.

Finally, tartaric acid presented a different behaviour for each production system: AGROCOM maintained initial level constantly; AGROMAN presented a peak at 4 days and then a decreasing trend; SUBST decreased until 8 days then remained constant; and CONV decreased over time reaching the lowest recorded values. At 14 days AGROMAN fruits showed a higher tartaric ac. content, almost double, than CONV. Regardless the system, fumaric acid remained constant overtime, with with no significant differences (data not shown).

Also, Amal et al. (2010) investigated the postharvest performance of ‘Festival’ strawberry (conventionally grown) at 0°, confirming a decreasing trend for TA overtime. As reasonable explanation, they assumed that the enzymatic reaction of respiration resulting in a consumption of organic acids. Also Asrey and Jain (2003) found a decreasing TA during 9 days storage of ‘Chandler’ strawberries while in Nunes et al (2002) TA didn't change significantly during two weeks of storage. Moreover, Koyuncu and Dilmaçünal (2010) reported a decreasing trend of citric, malic and fumaric acid during 10 days at 0°C in 'Dorit' and 'Selva' strawberries. Citric and Malic acid are the most abundant organic acid in Strawberries (Holcroft and Kader, 1997).

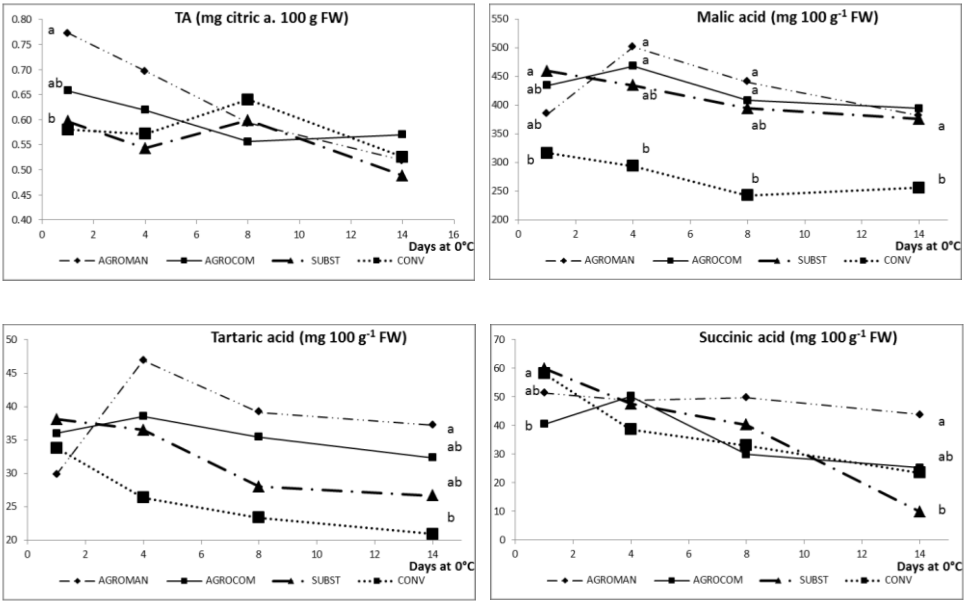


Figure 3. – Effect of the production system on TA and organic acids of strawberry fruits stored 14 days at 0°C. Per each time, letter indicates homogeneous group of means (Tukey, P<0.05)

Total soluble solids and sugars content evolutions are reported in Fig.4. At time zero, CONV fruits showed lower TSS content than AGROCOM and SUBST which remained constant during the storage varying of 0.5-1.0% in respect to the initial values but with no significant differences except than at 8 days. The TSS content has been strictly linked to the respiratory activity of produce in the study of Ayala-Zavala et al. (2004). They found out that the low respiration rate, diminished by cold temperature, may help to conserve the soluble solids concentration in strawberry fruits. Also, Amal et al. (2010) have reported a constant percentage of TSS measures in strawberry 'Festival' during 8 days at 0°C, while at 14 days a decrease occurred.

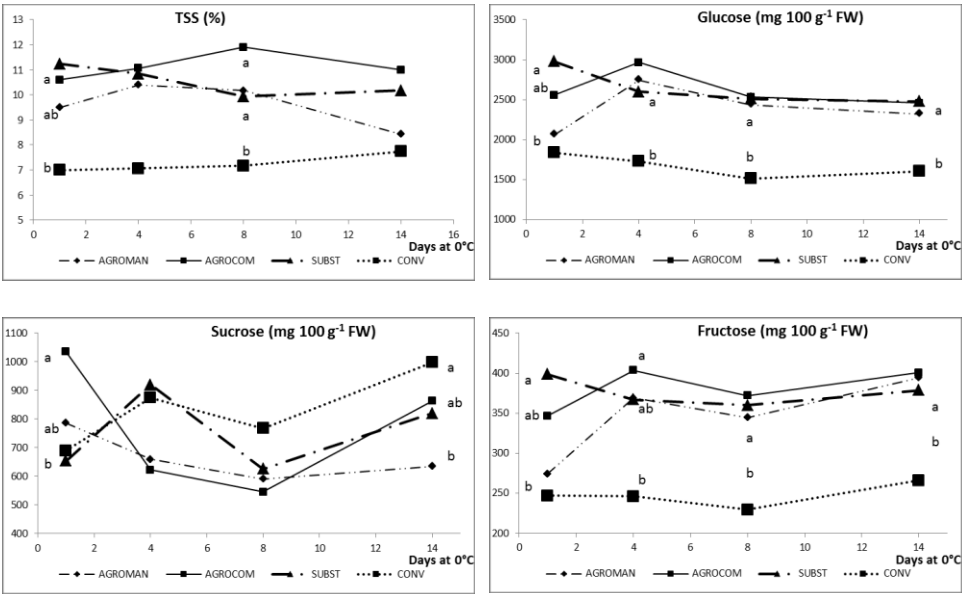


Figure 4. – Effect of the production system on TSS and Organic sugars of strawberry fruits stored 14 days at 0°C.Per each time, letter indicates homogeneous group of means (Tukey, P<0.05)

Glucose and fructose showed quite similar profiles of TSS over time. The initial glucose and fructose contents were stable in all the systems, with the exception of AGROMAN strawberries that, at 4 days, presented higher glucose and fructose contents in respect to their values at harvest. These augmented levels of glucose and fructose remained constant.

Generally conventional fruits showed lower glucose and fructose content than organic fruits, explaining the difference in TSS, even though at the end of the storage they showed higher sucrose content than AGROMAN with SUBST and AGROCOM showing intermediate value.

Strawberries vitamin C and ascorbic acid contents maintained initial concentrations (Fig.5); with the exception of SUBST, that presented a small increase, over time. AA and total vitamin C over time were consistently lower in CONV fruits than in organically managed systems, with some difference among these that were lost during storage. Indeed, dehydroascorbic acid increased at 4 days, and after that time, it kept to rise only in CONV strawberriesresulting significantly higher than for the organic one after 14 days.

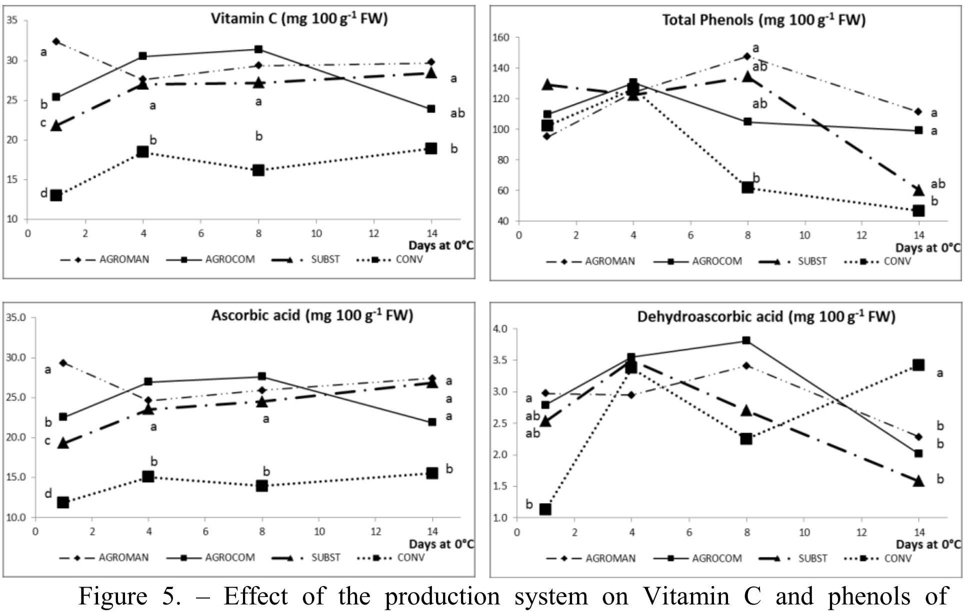


Figure 5. – Effect of the production system on Vitamin C and phenols of strawberry fruits stored 14 days at 0°C. Per each time, letter indicates homogeneous group of means (Tukey, P<0.05)

As for phenols, 4 day of storage, the total phenols values increased in respect their initial values for all the systems except for SUBST where they were stable up to 8 days and then markedly decreased to about 50% of their initial value. In CONV strawberries a huge decrease was observed already after 8 days, being significantly lower than AGROMAN and of AGROCOM and AGROMAN after 14 days.

A citric acid depletion during strawberries 'Camarosa' storage has been explained as a result of senescence processes (Sanz et al., 2002). While for tartaric acid no clear trend in tartaric acid fluctuations has been also reported by Koyuncu and Dilmaçünal (2010) as well as in this experiment.

During storage at 0°C (90-95%RH) relevant changes of the internal quality of strawberries in respect the initial values have been reported. This result indicates that the oxidative conditions which normally are faced during perishable produce storage had a much evident effect on CONV strawberries under reported experimental circumstances. It is possible that this major exposure to adverse conditions increased the ascorbate peroxidase activity which contributes to remove hydrogen peroxide but also to oxidise the ascorbate pool.

Phenol depletion in CONV confirmed what already observed in DHA trend, that indicates that conventionally grown strawberries were more exposed to oxidation processes which have consumed antioxidant compounds. Moreover also phenols in SUBST decreased over time, while AGROCOM and AGROMAN maintained the initial values clearly showing an effect of pre-harvest systems and the phenolic metabolism. Decreased phenol contents were also find in untreated-air strawberry 'Camarosa' during 12 days of storage (Allende et al., 2007). Indeed, total phenolic compounds were found increased continuously in Strawberry 'Chandler' during storage at 5°C and 10°C while they maintained a constant values when stored at 0°C (Ayala-Zavala et al., 2004). Also Piljac-Žegarac and Šamec

(2011) confirmed constant phenolic contents in strawberry during 9 days of storage at 4°C.

Sensorial attributes

Results of sensorial evaluation over time are reported in Fig.6. Both appearance and color decreased at 8 days, regardless to the system, reaching the minimum of the acceptability (3) at 14 days.

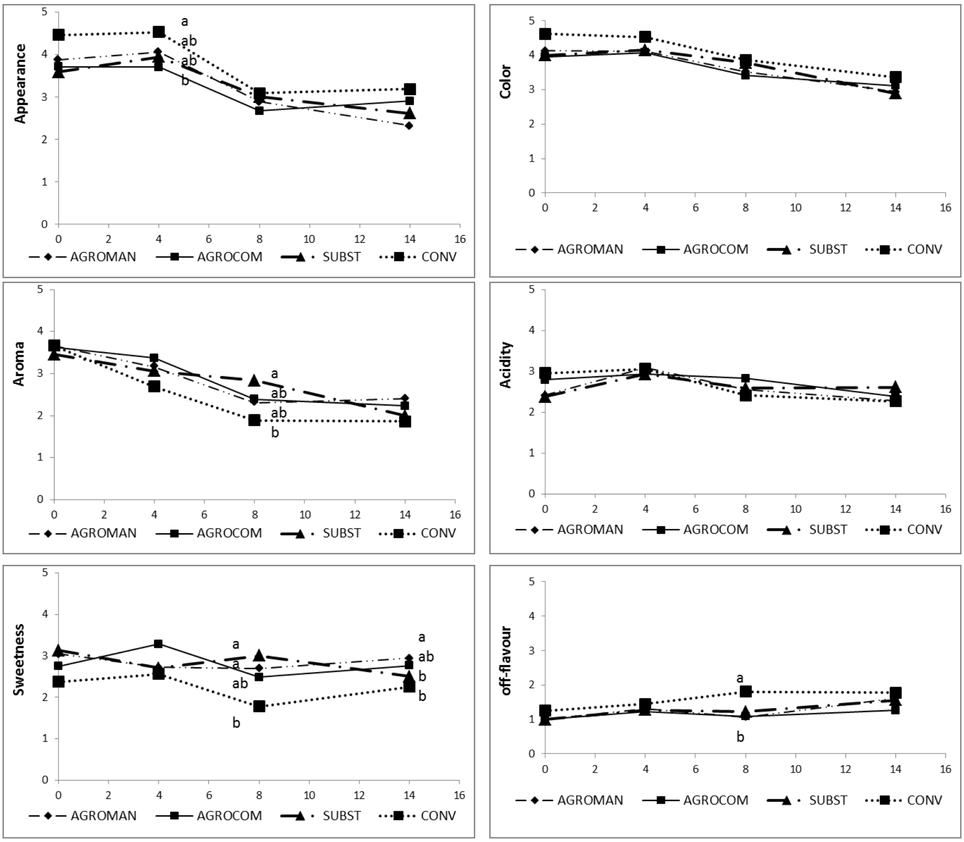


Figure 6. – Effect of the production system on Sensorial attributes of strawberry fruits stored 14 days at 0°C.Per each time, letter indicates homogeneous group of means (Tukey, $P<0.05$).

Appearance and color scores of CONV strawberries were slightly higher than for all other systems but this results was only significant at 4 days for appearance.

Aroma decreased over time reaching values around 2; CONV berries got the lowest values at any panel time but with no significant differences with the other system, except than at 8 days when they received a score lower than for SUBST fruits. Acidity, presented very limited variations among 2 and 3 score values without any significant difference among treatments.

As for sweetness score, values were quite stable over time with CONV fruits showing the lowest score that was significantly different from SUBST at 8 and from AGROMAN at 14 days. Off-odor had a slight increase over time for all the system, in particular, in CONV fruits at 8 days was significantly lower than all the other systems.

Multivariate approach to data interpretation

A comprehensive description of the obtained results has been attempted by using a multivariate approach. The principal component analysis was attempted as multivariate exploratory techniques. Preliminary, redundant information in the quality dataset were reduced: as for colour parameters were considered Hue°, Chroma and L*, for vitamin C were included its individual components AA and DHA, whereas sensorial attributes which did not vary so much among production systems were not involved in the elaborations.

The principal component analysis for data at harvest and at 14 days are shown in Figures 7 and 8, respectively.

At harvest (Fig.7a), the first two components (F1 and F2) accounted for 62.3% of the total variability recorded.

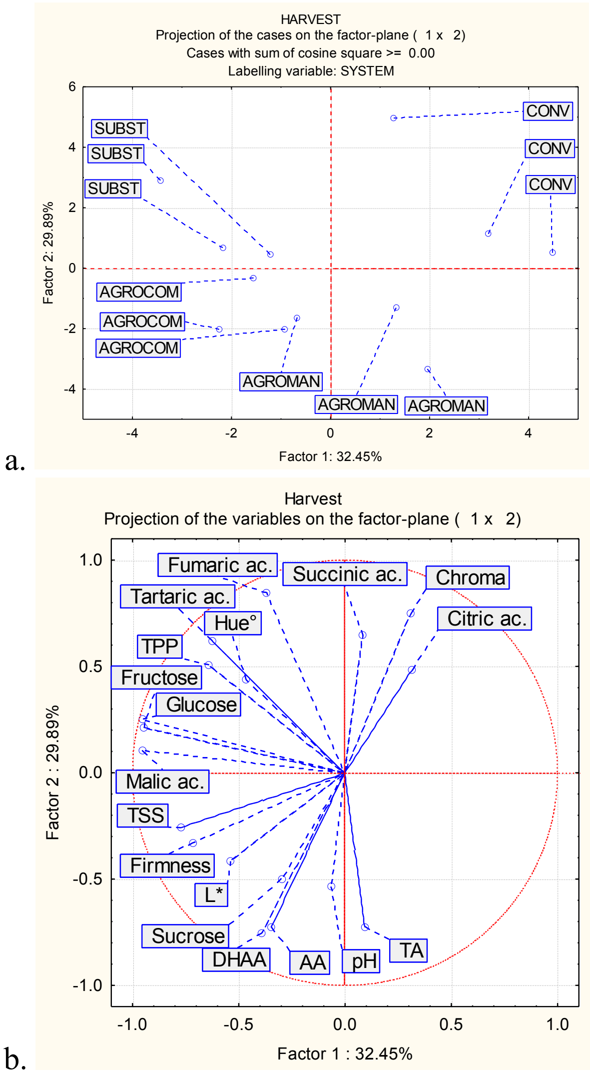


Figure.7 - Case scores (a.) and variables (b.) plots for the first two eigenvalues (x: Factor 1; y:Factor 2) after PCA of physical and chemical attributes of strawberries at harvest.

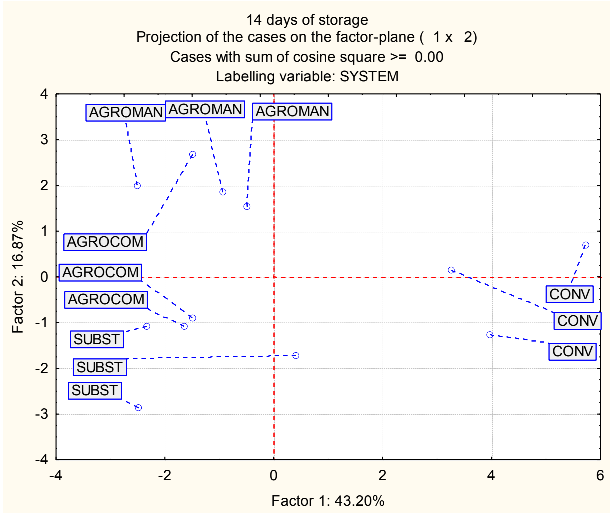
As observed, the first component (Factor 1) was able to clearly separate the data into two groups; conventional strawberries in the right-up quadrant and all the organic fruits in the left and in the bottom side. On the vertical axis, the second component (Factor 2) separated AGROCOM and AGROMAN strawberries, that scored negative y-values, from the SUBST and CONV ones, that were located in the upper quadrants. As contributes of each quality attributes at the two eigenvalues (Fig.7b), glucose, fructose, malic acid, TSS and firmness presented a negative correlation with Factor 1 and, at a minor extend, the same was observed also for phenol, tartaric acid, L* and Hue°.

Fumaric ac, Chroma, citric and succinic acids, presented a positive correlation with Factor 2, while TA, AA, DHA, pH, sucrose and L* showed a negative correlation in respect to the Y-axis. At 14 days, the first two components (Factor 1 and Factor 2) accounted for some 60% of the total variance. As observed for the initial analysis, the Factor 1 was able to clearly separate the data into two groups conventional strawberries in the right side and all the organic fruits in the left side (Fig. 8a).

The Factor 2 separated AGROMAN strawberries (above the positive value 1 of the y-axis) from the SUBST and CONV ones, with AGROCOM cases were in between the positive and negative part, closer on SUBST on the component 2.

The contributes of each quality attributes at the two eigenvalues at 14 days is plotted (Fig.8b). As described for initial quality, glucose, fructose, malic acid, firmness, L*, Hue°, TSS, tartaric acid and Phenols, presented a negative correlation with Factor 1.

A positive correlation is shown for pH, sucrose and DHA Concerning the vertical distribution in both the plots, citric, fumaric and succinic presented a positive correlation with factor 2, while TA showed a negative correlation in both the charts while most of the variables changed the orientation in respect to the Y-axis, if compared with the PCA analysis at harvest.

a.


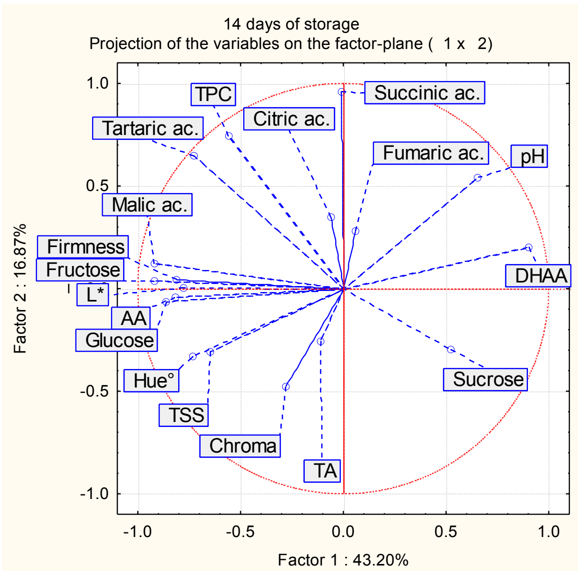
b.


Figure.8 - Case scores (a.) and variables (b.) plots for the first two eigenvalues (x: Factor 1; y:Factor 2) after PCA of physical and chemical attributes of strawberries after 14 days of storage.

At day 1 than day 14, both PCAs have elaborated new components that are able to separate organic and conventional systems along x-axis, and further grouping conventional and organic substitution systems in one cluster and the other systems (namely organic agroecological AGROMAN and AGROCOM) in a different cluster. The latter differentiation was less evident at 14 days than at harvest, indicating that the postharvest physiology of the strawberry fruits canceled or decreased the initial differences. This graphical representation of the data allowed immediate evaluation of the effects of the pre-harvest condition and of the postharvest performances of the strawberries quality attributes.

7.5 Conclusion

In conclusion, the production system affected the quality parameters of 'Festival' strawberries cultivated in greenhouse under Mediterranean climate conditions. Organically grown strawberries resulted in higher nutritional compounds at harvest and they better maintain their initial quality than conventional strawberries. A principal component analysis resulted in complete separation of conventionally grown strawberries from the other, but also it was able to identify relevant differences in the initial quality and postharvest attributes among the three organic systems. Possible explanations for the effects of production system on initial quality was due to the diverse mineralization rate of fertilizer and amendment supplied in the different systems. Nutrients mineralization rate in organic amendments is lower than in commercial organic fertilizers that, in turn, is lower than the nutrient solubility in mineral fertilizers. The secondary metabolites accumulation has been considered a plant stress response to moderate nutrient starvation. In this respect, organic substitution system presented intermediate characteristics between conventional and the other organic production systems in particular concerning initial values of vitamin C, postharvest evolution of phenols and titratable acidity and other organic acidis as observed in the principal component analysis. This differences reported at harvest were monitored during storage. Much of the initial diversity diminished due to the deterioration processes during postharvest. Moreover differently grown strawberries in similar have responded in different way at the storage of 14 days at 0°C.The oxidative conditions which normally are faced during perishable produce conservation had a much evident effect on conventional then organically grown strawberries under reported experimental circumstances. Organic strawberries showed better buffer capacity against oxidation due to secondary metabolites accumulated during pre-harvest period.

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8. Potentiality of NIR spectroscopy to discriminate among fruits from different production systems and to predict quality at harvest: case study on strawberries

Abstract

Strawberries as a non-climacteric fruit would beneficiate of non destructive and fast methods allowing to individuate the optimal harvest time. It is, in fact, essential to harvest strawberries at the optimum stage of ripening to achieve maximum initial quality, and ensure the best possibility to maintain initial quality level during postharvest storage. Nowadays, for consumer , other than the superior quality, also it is important to get authenticity proofs, in addition to certification. NIR methodology is promising to support the optimization of the postharvest management and hence to the implementation of tailored selection processes to match consumer requirements but it has been barely used for classification aims which may contribute to develop production systems authenticity test and validation. The aim of present study was to assess the potentiality of NIR spectroscopy to predict the internal quality attributes of organic and conventionally grown strawberries at harvest, and to discriminate fruits from 3 different production systems, two organically and one conventionally managed.

The overall results demonstrate that spectral information obtained with FT-NIR spectrometer multi-purpose analyser system scanning intact strawberries ‘Festival’, has excellent potential for the prediction of TSS (R^2=0.85; RMSEP=0.58), TA (R^2=0.86; RMSEP=0.09) and pH (R^2=0.58; RMSEP=0.15) of the strawberries produces under different fertility management. Ascorbic acid and

Phenols are confirmed much dynamic parameters to be reliable detected by NIR as also reported in literature. Moreover, the chemometric evaluation of strawberries spectra as a function of production systems performed was very satisfying classification using PLS-DA model, being able to correctly classify almost all samples with 1.00 sensitivity and 0.94 specificity in prediction. This results suggest that further test NIR methodology as a non-destructive technology on a wider dataset of produce, season, soils and production systems are necessary to assess the reliability of this technique as complementary method to the standard procedures for organic crop authentication.

8.1 Introduction

In the recent years people are getting aware that the site of origin and the method of production of the food is strictly linked to various health, social and environmental fallouts. This is guiding the increasing market demand of hygienic, certified and controlled food products. The consumer preferences have shifted to fruits and vegetables healthy and ecologically produced and toward processed foods having sustainable or social certifications and with sites of origin clearly reported on the label. This is the case of organic certified products that deserve increasing costumer willingness to pay (Adams and Salois, 2010; Krystallis and Chryssohoidis 2005). The growing demand and the premium price of organic produce, make actual the risk of frauds. Although certification system should guarantee a complete traceability all over-around the production steps, paper trail-based systems can be falsified. For consumer trust and defense it is important to get authenticity proofs, in addition to certification. (Capuano et al., 2013).

Recent scientific research is oriented towards developing various techniques to assist present certifications, traceability and audit procedures for food authenticity (Georgieva et al., 2014). Moreover, better forecasts of the initial quality of the product might contribute towards optimization of the postharvest management and hence to the implementation of tailored selection processes to match consumer requirements.

Mostly destructive instrumental techniques such as mass spectrometry and high pressure liquid chromatography (HPLC) are used for the analysis of the fruits and vegetables. These techniques are laborious, time consuming, costly and require chemicals reagents (Wu & Sun, 2013). Modern research is exploring fast, effective, efficient and low impact ways to non-destructively analyze the internal and external quality attributes of various food products, among which NIR spectroscopy (Oliveira et al., 2014; Sirisomboon et al., 2012; Cen and He 2007) and hyperspectral imaging are widely used (Puangsombut et al., 2012; Lorente et al., 2012).

The value of NIR technology for authenticating food products and determining their quality and shelf-life relies in the fact that every sample has a fingerprint NIR spectrum which consists of a unique and characteristic pattern of radiation. Two samples having very similar spectra, also possess very similar chemical and physical composition. Differences between spectra, by contrast, indicate that the samples are physically and chemically different (Workman and Shenk, 2004).

In the NIR range, the light is energetic enough to bring induce atom vibration. These vibrations absorb some light and reflect the rest in a scattering form. NIR spectroscopy detects fundamental vibration (overtones) between two different atoms (i.e. carbon oxygen chemical bond) in the reflected light. NIR spectra reports the amount absorbed in wavelength versus reflectance intensity chart.

Especially in the case of fresh horticultural produce Vis/NIR spectroscopy has a large scope and great interest is paid to actual applications. Many manufacturers of on-line grading lines have now implemented NIR systems to measure several quality attributes (Nicolai et al., 2007).

Strawberries as a non-climacteric fruit would beneficiate of non destructive and fast methods allowing to individuate the optimal harvest time. It is, in fact, essential to harvest strawberries at the optimum stage of ripening to achieve maximum initial quality, and ensure the best possibility to maintain initial quality level during postharvest storage (Sturm et al., 2003). Early picking does not guarantee maximum quality attributes in terms of taste, color and texture, whereas late harvest may lead to soft berries, more sensitive to physical damage and eventually to microorganism attacks during handling and storage (Kader, 1991).

Vis/NIR approach has been successfully applied to develop theoretical models for predicting strawberry quality attributes concerning color (Giovannini et al, 2014; Sanchez et al., 2012), firmness (Liu et al, 2014; Sanchez et al., 2012;

Tallada et al., 2006), bruise detection (Nagata et al, 2006), SSC (Liu et al, 2014; Giovannini et al, 2014; Guo et al., 2013; Aday et al., 2012; Sanchez et al., 2012; El Masry et el., 2007; Ito, 2002), glucose, sucrose and fructose (Nishizawa et al. 2009), pH (El Masry et el., 2007), water content (Aday et al., 2012; El Masry et el., 2007) and titratable acidity (Sanchez et al., 2012).

Furthermore, the same technique was applied for classification study confirming Vis/NIR ability to discriminate among strawberries at different ripeness stages (Liu et al., 2014; El Masry et al., 2007) and cultivars (Liu et al, 2014; Sanchez et al., 2012; Kim et al., 2009).

Few researches investigated that Vis/NIR methodology might be useful to develop prediction models based on spectra of organic and conventionally grown strawberries together. Moreover, this methodology has been barely used for classification aims which may contribute to develop production systems authenticity test and validation (Śmiechowska, 2007). To this aim, the potentiality of NIR methodologies have been investigated to discriminate organic Australian wine (Cozzolino et al., 2009) and organically and conventionally grown asparagus (Sanchez et al., 2013). Both the papers have reported successful NIR potentiality in production systems discrimination. In the latter study models constructed using partial least squares 2-discriminant analysis (PLS2-DA) correctly classified 91% of samples by growing method using a Vis/NIR spectrophotometer in the range 400–1700 nm. The wavelength range between 1315 nm and 1460 nm, mainly related to cellulose content, appeared to be especially relevant for the classification of asparagus by conventional vs. organic method (Sanchez et al., 2013).

The aim of present study was to assess the potentiality of NIR spectroscopy to predict the internal quality attributes of organic and conventionally grown strawberries at harvest, and to discriminate fruits from 3 different production systems, two organically and one conventionally managed.

8.2 Material and Methods

Cultural practices and plant material

Fruits from 3 different farming systems were used for this study. 2 organic systems differing by the management of the fertilization, (SUBST and AGROMAN) and one conventional SUBSTITUE (S), consisted in an organic input substitution widely adopted in organic production system, especially in greenhouse horticulture, which mimics conventional agriculture by substituting conventional agrochemicals with allowable organic products; AGROMAN (M), is characterized by the use of a cover crop mixture (based on legume species) and mature manure (from organic husbandry); CONVENTIONAL (C), was a conventional farming system based on 100% mineral fertilizers. 'Festival' strawberry roots, certified as organic from the same nursery (Vivai f.lli Zanzi – Ferrara, Italy) were used both for organic and conventional productions.. In all the systems, strawberries were transplanted on late September 2014 and in May 2015, fruits of different maturity level (Based on berry surface color: the white fruits, 1/2 pink-red colored; 2/3; 3/4 and the full red), visually appraised, were hand harvested randomly from heterogeneous sampling areas per each production system for a total 210 fruits, 100 for organic M, 90 and S and 30 for C system. Samples were quickly delivered to the postharvest laboratory at the University of Foggia-After the acquisition of three spectra for each intact berry, pH, TA, TSS were analyzed for each single fruit (from M, S and C samples), moreover ascorbic acid and phenols were determined for the organic strawberries M and S.

Chemical quality characteristics

Each fruit was squeezed and the obtained juice drops were used for direct readings of total soluble solids (TSS%) content with a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan).

Total acidity (TA) and pH were measured on 5 g of squeezed juice for each replicate, using an automatic titrator (TitroMatic CRISON, Spain) with 0.1 mol L^{-1} NaOH solution up to final pH of 8.1 and reported as percentage of citric acid per 100 g.

Vitamin C (Vit-C), L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), content was determined as described by Zapata and Dufour (1992) with some modifications (Gil et al., 1999). Total phenols were determined according to the method of Singleton and Rossi (1965).

Antioxidant assay was performed following the procedure with DPPH described by Brand-Williams et al. (1995) with minor modifications. The diluted sample, 50 μL, was pipetted into 0.950 mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after overnight incubation using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). Trolox was used as a standard and the antioxidant activity was reported in mg of Trolox equivalents per 100 g of fresh weight (mg TE 100 g-1 FW).

Total phenol content was determined on 5 g of fruit tissue extract according to the method Singleton and Rossi (1965). The content of total phenols was calculated on the basis of gallic acid calibration curve and expressed as mg gallic acid kg^{-1} of fruits fresh weight.

NIR Spectra acquisition:

The analysis was performed on fruits at room temperature (~20 °C). Three scans per sample were acquired by using a FT-NIR spectrometer (Multi- Purpose Analyzer MPA , BrukerOptics, Ettlingen, Germany). Reflectance mode was used during spectral acquisition, over the absorbance range 12500 cm^{-1} to 3600 cm^{-1} (Sphere macrosample resolution 16 cm^{-1}; scanner velocity 10 kHz; sample scan time 64 scans; background scan time: 64 scans). The instrument is equipped with a high energy air cooled NIR source (20 W tungsten-halogen lamp) and uses a permanently aligned and highly stable RockSolid interferometer. The interferometer has a wave number reproducibility better than 0.04 cm^{-1} and a wave number accuracy better than 0.1 cm^{-1}.

For prediction purpose, the spectra were firstly elaborated with the OPUS Software (Version 7,2m Bruker Optik GmbH 2012) in order to screen the most relevant wavelength ranges and mathematical preprocessing, and then imported in Matlab (Version R2015a, The Mathworks Inc., Natick, MA, USA). Classification analysis was performed directly in Matlab. A total of 630 spectra were obtained (3 spectra x 90 berries x 2 systems plus 3 spectra x 30 berries x 1 system).

Spectra Pretreatment

Prior to pretreatment of the data, the spectra were analyzed with a PCA (Principal component analysis) central model to identify and eliminate defective spectral outliers (Massart et al., 1998; Naes et al., 2002).. From the plots of the PCs all the points lying outside the 95% confidence sphere were eliminated before dividing the data sets into a calibration and a prediction set. On the calibration spectra various pretreatments were applied individually and in combination, such as smoothing, derivatives, MSC, baseline and SNV.

The accuracy of calibration depends on the model errors, namely, root mean square error for cross validation (RMSECV) and root mean square error for prediction(RMSEP used for internal or external validation, respectively. These are defined as follows: $RMSEP = RMSECV = \sqrt{\frac{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2}{n_p}}$; where, $\hat{y}_i$ is the predicted value of an attribute in fruit number i; y_i is the measured value of an attribute in fruit number i; n_p is the number of validated cases. Model can be expressed by the predicted values $y_i \pm 1.96\, x\, RMSEP$ (Nicolai, 2007). This value gives the average uncertainty that can be expected for predictions of future samples in the 95% confidence interval. The number of latent variables in the calibration model is typically determined as that which minimizes the RMSECV or RMSEP. Another useful statistic is the R^2 value. It essentially represents the proportion of explained variance of the response variable in calibration (R_c^2), cross validation (R_{cv}^2) or external prediction (R_P^2) sets.

Partial Least Square Regression (PLSR)

PSL SIMPLIS algorithm was used to create predictive models for the desired parameters within the optimal wavelength ranges. The measured parameters included soluble solids content (TSS), pH, titratable acidity (TA), phenols, dehydroascorbic acid (DHA), Ascorbic (AA) and Vitamin-C. In case of PLSR the covariance is maximized between the linear functions of the spectral variations X and the corresponding defined value Y to be correlated. Default setting of the venetian blinds cross validation was used. For each model the performance of the PLSR was judged by comparing the difference between the root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV), which should be minimum for a better model. Then the same preprocessing technique was applied on prediction set to test the model in prediction. Model with highest R^2 both in calibration and prediction, and lowest RMSEP were preferred.

Development of classification model

The PLS-DA model is a supervised algorithm based on the relation between spectral intensity and sample characteristics; in our case using spectral variations as (X) and 3 established categories, corresponding to the system of production as (Y), trying to maximize covariance between the two types of variables.

PLS2-DA is therefore performed using an exclusive binary coding starting by PLS regression, which uses M spectral variables as predictors and q dummy variables (0 or 1) assigning value to the class to be discriminated in each model and value 0 for the other.

During the calibration process, the PLS-DA method is trained to compute the "membership values", one for each class; the sample is then assigned to one class when the value is above a specific prediction threshold. (Musumarra et al., 2005; Liu et al., 2007). Spectra subjected to the various pre-processing techniques were used to construct the model, using random cross-validation.

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

Also in this case the best model was also tested in prediction, preprocessing the spectra as for the calibration model. Confusion matrix in which the diagonal objects represent the correctly classified objects can be a good indicator of the classification model performance.

It also leads to the development of valuable indices such as non-error rate (NER) or classification rate which represents the percentage of the correctly classified samples and is the average of the sensibility calculated over the classes as follow: NER= $\frac{\sum_{a=1}^{A} n_{aa}}{n}$, where n represents the number of objects.

8.3 Results and discussion

Composition of strawberry

In Table 1 are reported the mean values of each measured attribute for each production system. The high standard deviation observed in some cases is due to the fact that fruits were harvested at different maturity stages in order to enlarge the interval of variation of the calibration models.

Table 1 – Quality attributes of strawberries fruits (Average and SD:stand. dev.) by production systems

Parameters	**M**	**SD-M**	**S**	**SD-S**	**C**	**SD-C**
TA (% citric ac.)	1.07	*0.24*	1.05	*0.23*	1.07	*0.23*
TSS (%)	8.0	*1.4*	8.1	*1.4*	12.2	*3.7*
pH	3.33	*0.52*	3.47	*0.75*	3.64	*0.24*
Phenols (mg $100g^{-1}$)	292.5	*29.0*	309.2	*46.5*		
Vit-C (mg $100g^{-1}$)	48.7	*7.5*	48.4	*8.7*		
DHA (mg $100g^{-1}$)	24.6	*7.5*	30.6	*11.0*		
AA (mg $100g^{-1}$)	43.7	*6.7*	31.8	*5.9*		
DPPH (mg TE $100g^{-1}$)	308.2	64.3	387.7	43.8		

Prediction of internal composition

The first screening made with OPUS software already suggested that some parameters, as AA and antioxidant activity could not be predicted based on NIR spectra; these finding were also confirmed by further analysis of the data, and therefore results are not reported. In Tab.2 the prediction results in term of R^2 and Root Mean Error for calibration and prediction are shown.

Table 2- Pretreatments performance in the prediction of strawberries quality attributes

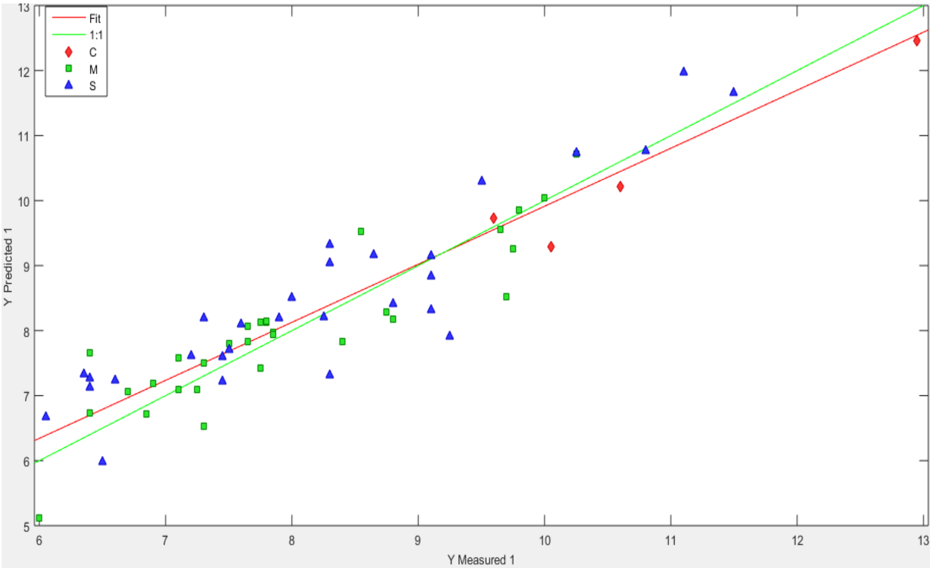
Parameter	Pretreatment	R_c^2	*RMSEC*	R_{cv}^2	*RMSECV*	R_p^2	*RMSEP*
TSS	SNV	0.92	0.62	0.90	0.70	0.81	0.66
	Smoothing S. Golay	0.89	0.73	0.86	0.81	0.86	0.78
LVs=7	1st Derivative, MSC(mean)	**0.94**	**0.54**	**0.91**	**0.67**	**0.85**	**0.58**
WR (cm^{-1})= 9401-4597	2nd Derivative, MSC(mean)	0.92	0.60	0.83	0.91	0.67	0.90
	Baseline, SNV	0.93	0.54	0.83	0.89	0.68	0.88
pH	SNV	0.75	0.10	0.76	0.11	0.70	0.11
	Smoothing, MSC(mean)	0.73	0.10	0.69	0.11	0.68	0.11
LVs=7	1st Derivative, MSC(mean)	**0.86**	**0.07**	**0.80**	**0.09**	**0.75**	**0.09**
WR (cm^{-1})= 7507-6094 5454-4242	2nd Derivative, MSC(mean)	0.54	0.14	0.35	0.16	0.56	0.14
	Baseline, SNV	0.75	0.10	0.71	0.11	0.71	0.11
TA	SNV	**0.66**	**0.12**	**0.60**	**0.13**	**0.57**	**0.15**
LVs=5	Smoothing S. Golay, MSC(mean)	**0.64**	**0.12**	**0.60**	**0.13**	**0.56**	**0.15**
WR = 7507-4242 cm^{-1}	1st Derivative, MSC(mean)	**0.65**	**0.12**	**0.57**	**0.13**	**0.58**	**0.15**
	2nd Derivative, MSC(mean)	0.59	0.13	0.44	0.14	0.56	0.15
	Baseline, SNV	0.60	0.13	0.50	0.14	0.66	0.13
Phenols	SNV	0.44	22.93	0.41	22.50	0.39	20.06
	Smoothing S. Golay	0.45	21.78	0.42	22.35	0.45	19.46
LVs=4 WR (cm^{-1})= 6103-5446 4428-4242	1st Derivative, MSC(mean)	0.50	20.78	0.44	21.91	0.42	19.99
	2nd Derivative, MSC(mean)	0.45	21.74	0.41	22.51	0.43	19.52
	Baseline, SNV	0.45	21.74	0.42	22.30	0.37	20.58
DHA	SNV	0.52	5.99	0.41	6.70	0.41	6.87
	Smoothing S. Golay	0.13	8.14	0.07	8.50	0.09	8.92
WR (cm^{-1})= 9404-6094 5454-4597	1st Derivative, MSC(mean)	0.53	5.94	0.42	6.63	0.39	7.01
	2nd Derivative, MSC(mean)	0.48	6.27	0.31	7.27	0.35	7.24
	Baseline, SNV	0.62	5.32	0.41	6.71	0.43	6.84
Vit-C	SNV	0.26	6.18	0.23	6.35	0.33	6.77
	Smoothing S. Golay	0.40	5.60	0.35	5.83	0.25	7.39
WR (cm^{-1})= 7753-6094	1st Derivative, MSC(mean)	0.47	5.24	0.38	5.68	0.49	5.91
	2nd Derivative, MSC(mean)	0.10	6.85	0.08	6.93	0.12	7.70
	Baseline, SNV	0.30	6.03	0.20	6.48	0.32	6.91

LVs= latent variables; WR=wavelength ranges (cm^{-1})

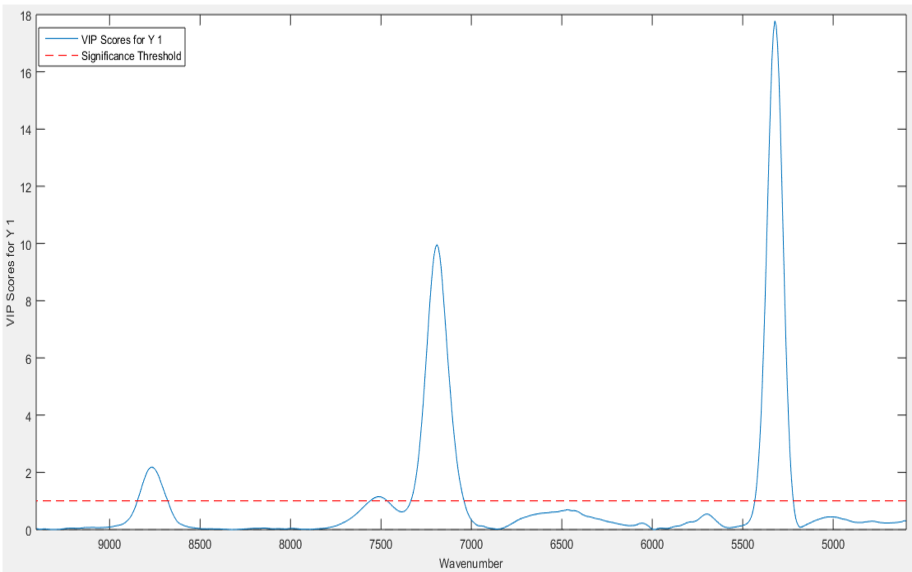
Particularly, the result of the effect of different preprocessing on model performance are reported for each parameter. The calibration curve and the results of prediction of TSS on the external data are reported in Fig.1a. As can be observed, the production system (marked in different colors) did not affect the model, and TSS were well predicted regardless the production systems and despite the higher soluble solids contents measured in C than in the other classes (Tab.1). By evaluating the Variance Importance in Prediction (VIP) scores by wavenumber shown in Figure 1b it is possible to evaluate which part of the signal is more relevant for the prediction. Three peaks can be observed in the VIP graph around 1142nm, 1388nm and 1869nm. Results on pH are reported in Fig.2; the prediction set resulted was modeled for C and M classes, while for S class, slightly the points around 3.3 pH values were overestimated. As resulted from the VIP score (Fig.2b) the most important wavelengths for the model are in the two main ranges,1369 - 1408 nm and 1821-1923nm.

As for TA (Fig.3) the prediction didn't fit as well as the calibration, mainly the model has overestimated actual values for M class. However, difference between predicted and measured values are less than 0.3 percent points in the worst case, that might be an acceptable result for such a variable parameter as the TA of the berry fruits collected at any maturity stage. In this case there are 2 main peaks one at 1388 nm and the second at 1869 nm (Fig, 3b).

In the scientific literature are reported previous attempts to predict internal quality parameters of strawberries. Liu et al. (2014) have well predicted TSS from multispectral imaging (405–970 nm) of strawberries with R^2 of 0.83 and SEP of 0.573, which are comparable to our results.

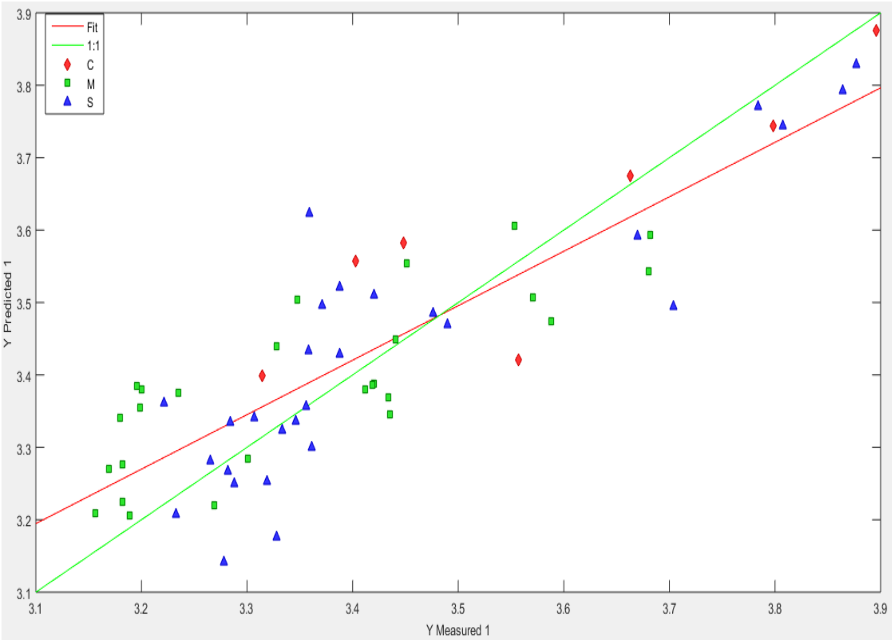


a.

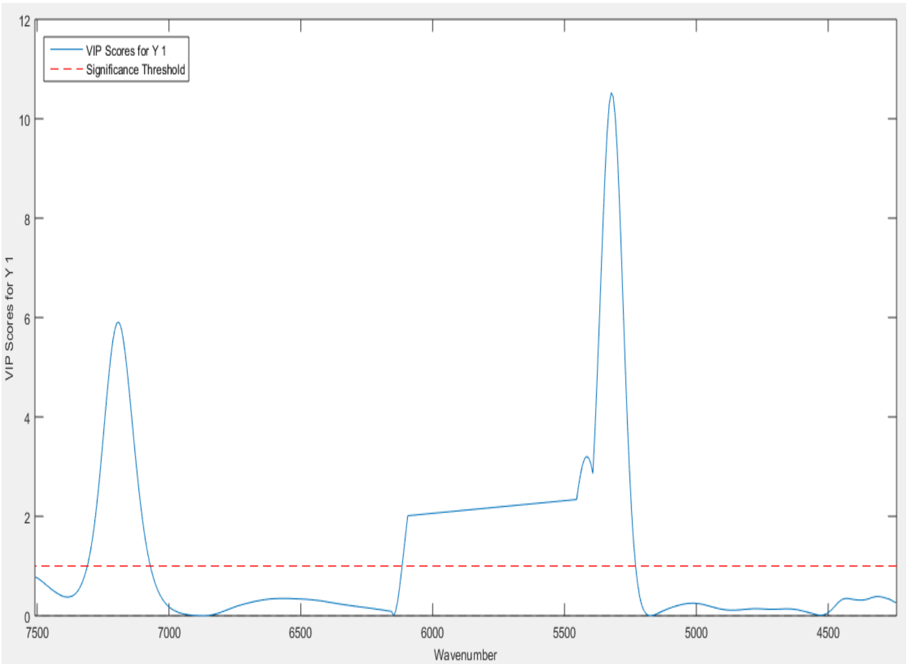


b.

Figure 1 –Measured versus predicted values of TSS for the external prediction set (a) and VIP Score by wavenumber/wavelength plot (b).

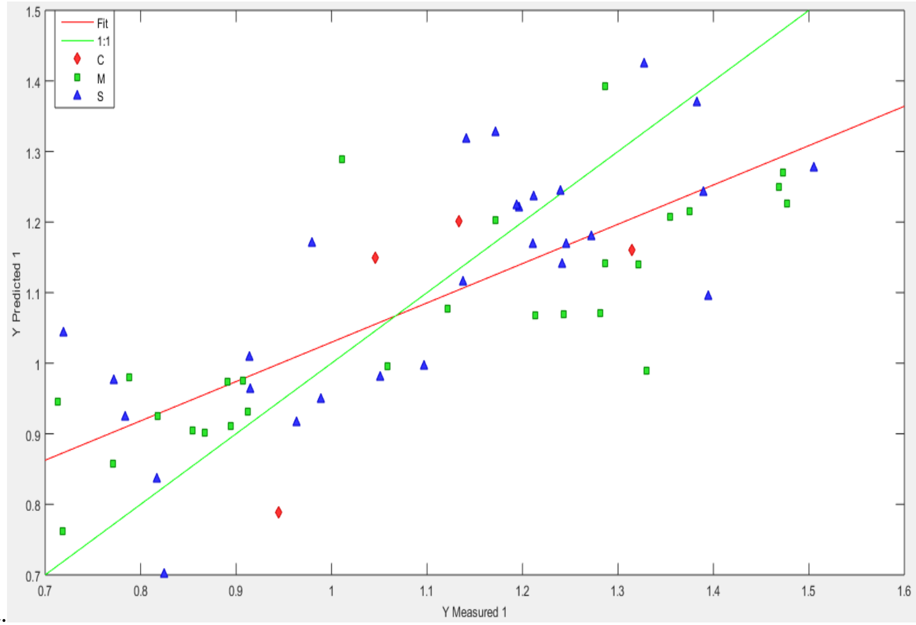


a.

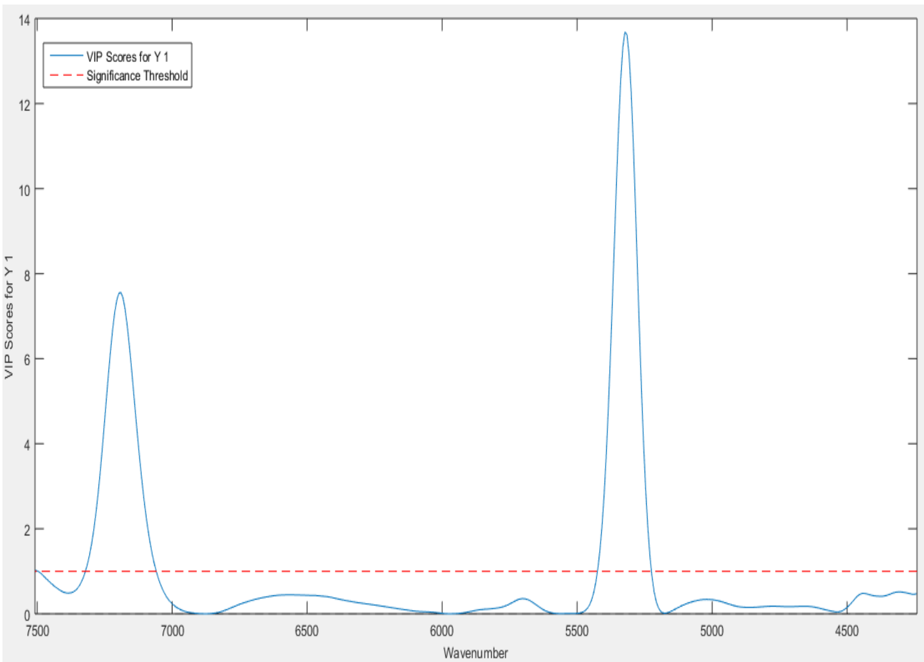


b.

Figure 2 – Measured versus predicted values of pH for the external prediction set (a) and VIP Score by wavenumber/wavelength plot (b).



a.



b.

Figure 3 – Measured versus predicted values of TA for the external prediction set (a) and VIP Score by wavenumber/wavelength plot (b).

ElMasry et al. (2007) collected strawberries at different ripeness stages from local retail stores and they acquired hyperspectral images in the range of 400–1000 nm. On the whole spectra PLS model was able to predict TSS with R^2 of 0.85 and SEP of 0.184, and pH with R^2 of 0.87 and SEP of 0.129, obtaining therefore a lower error for TSS but a much higher error for pH, if compared to our findings. Sanchez et al. (2012) used NIR spectra in the range of 1600-2400 nm, and LOCAL algorithm with PLSR for prediction over 9 strawberry cultivars using reporting for TSS R^2 of 0.69 and SEP of 0.88, for pH R^2 of 0.40 and SEP of 0.11 and TA with R^2 of 0.65 and SEP of 0.07. Nishizawa et al. (2009) applied NIR spectroscopy (700-925 nm) to determine TSS in 'Akihime', 'Benihoppe', and 'Sachinoka' strawberries reporting R^2 0.86 and SEP 0.9%. Also the TSS of 'Akihime' and 'Toyonoka' strawberries were successfully predicted by Nagata et al. (2005) and Ito (2002) confirming soluble solids a much studied and well predicted attributes other than pH and TA. As for other internal quality parameters, Giovannini et.al. (2014) tried to predict ascorbic acid and total anthocyanins, in addition to TSS and AA for 6 varieties, but only TSS could be predicted with sufficient accuracy (SEP of 0.53-0.89). Very few study included other parameters, and no one phenol content. Results of the present study explored the possibility to predict nutritional components in addition to maturity index, indicating that phenols and vitamin C cannot be predicted with high accuracy, but that NIR can be used to predict some concentration ranges for these compounds.

Classification model to discriminate among fruits from different production systems

For the classification of the d 3 classes of strawberries obtained with 3 different production systems, partial least square discriminant analysis (PLS-DA) was carried out. Out of a total of 210 samples (30 in class C, 90 in class M, and 90 in class S) the PLS-DA was performed using a calibration set of 145 samples (60 for M and S and 25 for C) and a validation set of 65 samples (30 for M and S and 5 for C). Different pretreatments were applied also in this case, among which Baseline used with MSC (mean) helped the values of sensitivity and specificity to get closer to 1. The values of sensitivity and specificity for calibration, validation and prediction set are reported in Tab.3, and shon in Figure 4 for the prediction set.

Table 3. Sensitivity and Specificity values of PLS-DA classification of fruits in the 3 classes for calibration, CV and prediction set.

Classes		**Cal**		**CV**		**Pred**	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
1	M	1.000	1.000	1.000	0.992	1.000	0.983
2	S	0.967	0.988	0.950	0.941	0.967	0.943
3	C	0.983	0.988	0.917	0.953	0.967	0.971

The model allowed to obtain sensitivity and specificity close to 1 for all production system, with a “Non Error Rate” of 98.3, 95.57 and 97.8, in calibration, cross validation and prediction, respectively. This parameter is the average of the sensibility calculated over the classes and gives an overall idea of the goodness of the classification.

In the literature classification studies have investigated different methodologies to individuate rapid determination of strawberries cultivar or maturity level. Both Fourier transformed infrared spectra of leaves and fruits (Kim et al., 2009) and NIR technology with PLS-DA have succeeded in cultivar classification (Sanchez et al., 2012); multispectral images in the Vis/NIR with different models (PLS, SVM; BPNN) allowed to predict strawberries ripeness stage (Liu et al., 2014). This results was achieved also by texture analysis with the grey-level co-occurrence matrix based on hyperspectral images and discriminant analysis (ElMasry et al., 2007).

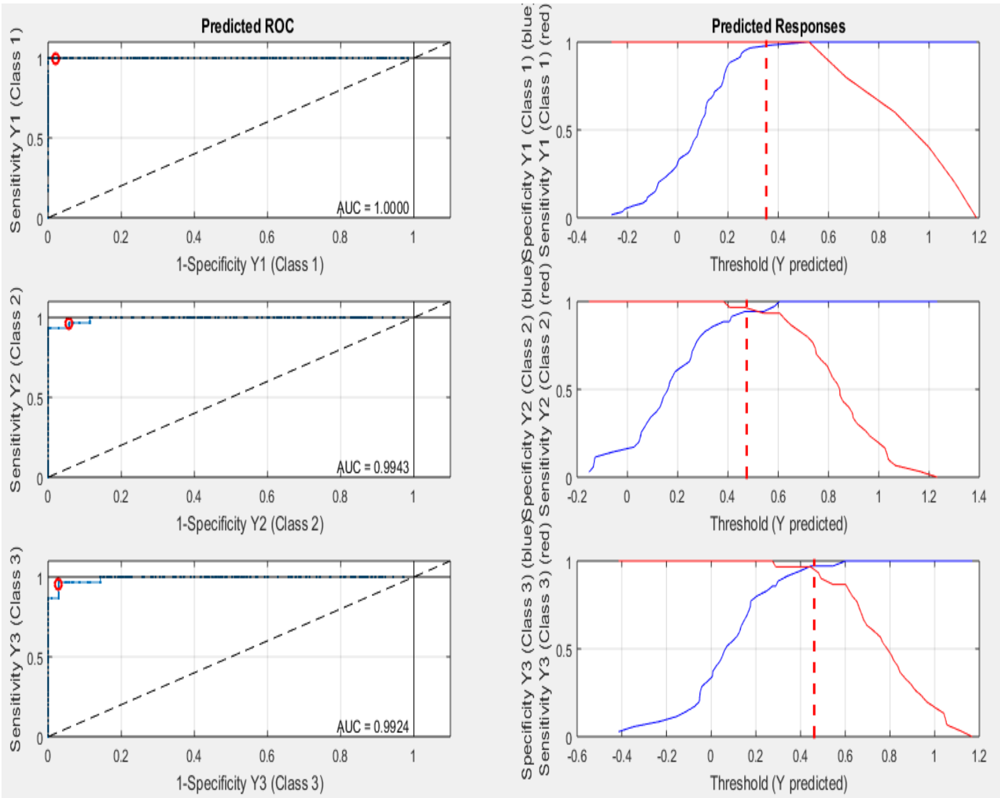


Figure 4. Specificity (blue) and sensitivity (red) for PLS-DA classification of strawberries into 3 classes. (Class 1=M; Class 2=S; Class 3= C)

Nonetheless the classification/discrimination of origin place and methods of fruit production is achieving increasing interest in scientific publications. Two recent studies has evaluated two different approached at this issue, denoting the relevance of the food authentication question for organic strawberry fruits. The phytochemical metabolic profile of strawberries, by liquid chromatography coupled to high resolution mass spectrometry to have metabolomic fingerprint, was able to discriminate conventional and organic strawberries and red (ripe) and white (unripe) fruits (D'Urso et. al. 2015). Combining isotopic and chemical markers with canonical discriminant analysis was possible to discriminate completely the geographical origin of strawberries and, inside each area, the model allows to distinguish also the agricultural regime (Camin et al., 2011).

To our knowledge, there are no published studies in literature concerning the classification of strawberries according the sites of origin either the systems of production by using NIR spectroscopy.

This technology was evaluated for organic wine (Cozzolino, Cynkar, Shah, Dambergs, & Smith, 2009) and for asparagus (Sanchez et al., 2013) classification. As for wine, the 85% (57 organic and 115 non-organic, from 13 Australian regions), were correctly classified with PLS-DA (Cozzolino et al., 2009). In the study on asparagus (*Asparagus officinalis* L., cultivar 'Grande'), NIR reflectance spectroscopy and PLS2-DA correctly classified 91% of samples intact green asparagus as a function of growing method (organic vs. conventional), 100% of samples as a function of harvest month and 97% of samples by postharvest cold storage duration. Sanchez et al. (2013) concluded suggesting the incorporation of sensor with NIR technology in the horticultural industry to authenticate the organic vs. conventional origin of green asparagus.

In the present experiment, three production systems were correctly discriminated with more than 95% of sensitivity and more than 94% specificity showing promising results for further investigation of NIR methodologies to be used for organic discrimination. However the low number of system and production sites included in the analysis shall be considered a strong limitation to make generalizations of these results.

Worthy, in this experiment, NIR methodology was able to discriminate not only conventional and organically grown strawberries but also between two organic systems of production which were implemented on the same site, over the same soil, under the same unheated tunnel, with the same genetic material. The only difference consisted in the nutrient input which was discriminated with high accuracy. This results suggest to further test NIR methodology as a non-destructive technology on a wider dataset including more source of variation as diverse soil types and climatic conditions, a high number of cultivars and different agronomic practices, and different species in order to assess the real potential of this technique and its reliability as alternative or complementary method to the standard procedures for organic crop authentication.

8.4 Conclusion

The overall results demonstrate that spectral information obtained with FT-NIR spectrometer multi-purpose analyser system scanning intact strawberries

'Festival', has excellent potential for the prediction of TSS, TA and pH attributes of the berries produces under different fertility management. Ascorbic acid and Phenols are confirmed much dynamic parameters to be reliable detected by NIR as also reported in literature. Moreover, the chemometric evaluation of strawberries spectra as a function of production systems performed was very satisfying classification using PLS-DA model, being able to correctly classify almost all samples. Further interesting developments may in fact be aimed to the application of this algorithm to classify in real time the strawberries in a sorting line, or also to further develop a method to identify the authenticity of the production methods of strawberries production. Moreover, this approach can be implemented as a fast, clean methodology that allows producers to improve strawberries quality in the whole chain, facilitating the best harvesting time decision and the selection of clusters of different initial quality; and supports inspection body in the certification audit and in the identification of authentic production systems.

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Conclusion

Organic production system has the objective to achieve a high quality level with a rational use of natural resources Nowadays, organic agriculture is increasing in terms of invested surface and number of operators. This originated a wide set of solutions which, although valid with respect to the organic certification standards, still need scientific assessments concerning the claimed sustainability and high quality performances. A more in deep analysis of quality of organic products may be oriented to the comparison of their quality with conventional products grown in the same pedoclimatic conditions, also in term of the effect of pre-harvest practices on postharvest performances. In this respect, the implementation of agro-ecological practices and long term strategies for the soil fertility management in organic farming have been compared to the substitution approach (namely conventionalized system) in which the nutritional needs or lacks are treated “substituting” off-farm synthetic inputs with off-farm organic inputs allowed in the Regulation.

In terms of quantity two years of strawberries and one year of tomatoes production resulted in similar yield among the systems, with substitution systems producingmore than the agroecological systems, but without statistical evidence.

As for initial quality, different soil fertility management practices resulted in different quality at the harvest. For organic lamb’s lettuce dehydroascorbic acid and phenols resulted higher in SUBST than in the other two systems; also for breaker and pink tomatoes dehydroascorbic acid and phenols resulted higher in SUBST than in AGROCOM system, with AGROMAN in between. As for strawberries, AGROMAN and AGROCOM allowed to obtain fruits with better characteristics in terms of color and higher phenolic content than SUBST fruits, on an average of the two years of investigation. Moreover, for strawberries was possible to set up a comparison with conventionally grown fruits of the same variety, grown locally in the same pedoclimatic conditions. At harvest, organically grown strawberries resulted in higher ascorbic acid than conventional strawberries that resulted also in lower TSS, glucose and fructose than SUBST system.

For all the experiments, these differences reported at harvest were monitored during storage in refrigerated conditions. Much of the initial diversity diminished due to the deterioration processes during postharvest and the differences among organic production systems became less important over time, for all the studied products.

As for lamb's lettuce, AGROMAN and AGROCOM showed less quality changes over time than SUBST, whereas regarding the effect of packaging AIR conditions succeeded to maintain the initial quality attributes for a longer period in comparison to the modified atmosphere package that, at 11 day despite the production system, developed off-odors under the threshold of acceptability. In case of strawberries, the oxidative conditions which normally are faced during storage of perishable product had a much evident effect on conventionally then on organically grown strawberries under reported experimental circumstances. Organic strawberries showed better buffer capacity against oxidation due to secondary metabolites accumulated during pre-harvest period.

Possible explanations for these effects of production system on initial and postharvest quality have been related to plant stress response to moderate nutrient deficiency which was due to the diverse mineralization rate of fertilizer and amendment supplied in the three systems. Other possible factors shall be further investigated about the interaction among the exogenous organic matter decomposition, microbial community of the soil and the root uptake and signaling to sink organs of the plant. However, the balance between primary and secondary metabolic pathways seems to be an effective key to re-discuss the complex interaction of genotype, environment and agricultural practices which lead to different quality and postharvest performance of fresh fruits and vegetables. The need to improve the quality of food available in the world should orient agricultural practices to increase the nutritional composition of fresh fruits and vegetables and to enhance the shelf life expectations.

A contribute in this direction was attempted with the application of NIR methodology on intact strawberries 'Festival'. The spectral information obtained with FT-NIR spectrometer have been successfully modelled to predict strawberries TSS, TA and pH attributes. Phenols could be predicted with lower accuracy, while NIR was less promising for the prediction of antioxidant activity and Vitamin C, as also reported in literature. Moreover a PLS-DA analysis on spectral data allowed to correctly classify the fruits based on the production system, both among conventional and organic and between two organic systems.

Further interesting developments of this results may be in fact be aimed to the study of the applicability of this method to identify the authenticity of the production system of strawberries, by increasing the variability of the production factors (cultivar, location, growers, etc) into the model. Moreover this approach can be implemented as a fast, non destructive methodology that allows growers to improve strawberry quality in the whole production chain, supporting the individuation of the best harvesting time and the selection of clusters of different initial quality, also to be applied online on sorting machines.