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FACOLTA' DI AGRARIA
DIPARTIMENTO DI SCIENZE DELLE PRODUZIONI AGRARIE E ALIMENTARI
DOTTORATO DI RICERCA IN SCIENZE E TECNOLOGIE ALIMENTARI
XXV CICLO

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QUALITY AND TRACEABILITY OF TYPICAL MEDITERRANEAN FRUITS

PhD Thesis

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TRIENNIO 2010-2012

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INDEX

1. Review

1. Review

Qualità e tracciabilità sono il binomio vincente per lo sviluppo di un'agricoltura in chiave moderna, dove accanto alla tradizionale funzione economico– produttiva stanno progressivamente trovando spazio ulteriori funzioni riconducibili alla valenza territoriale, ambientale e sociale.

Nell'ultimo decennio la qualità è diventata funzione essenziale per garantire l'accesso al mercato e ad una efficace concorrenza verso un numero sempre crescente di paesi esportatori. La qualità è definita come l'insieme delle caratteristiche degli alimenti che portano alla soddisfazione dei consumatori e include proprietà sensoriali, vita di scaffale, sicurezza dei prodotti e proprietà funzionali.

I sistemi per la tracciabilità dei prodotti alimentari lungo le fasi della produzione, della trasformazione e della distribuzione svolgono un ruolo fondamentale nel garantire la sicurezza, la qualità e l'origine dei prodotti alimentari e sono di norma basati su un continuo "paper-trail" e su un'etichettatura adeguata. Tuttavia, le tecniche analitiche che consentono di determinare la provenienza di un alimento costituiscono un mezzo indipendente di verifica ed inoltre aiutano a dimostrare l'autenticità del prodotto, a combattere abitudini fraudolente e a controllare eventuali adulterazioni.

La capacità di certificare l' origine alimentare o l'autenticità di un alimento è di rilevante importanza economica per gli attori delle filiere agroalimentari in diversi paesi. Ad esempio, alcuni prodotti alimentari (a marchio bio e/o con certificazioni d'origine) possono fregiarsi dei riconoscimenti ricevuti in ambito CE e questo aggiunge valore a tali prodotti in termini di commerciabilità e di volumi di esportazione.

Il presente lavoro di tesi è stato sviluppato secondo questa prospettiva, ponendo l'attenzione sulla qualità e tracciabilità dei frutti tipici e loro derivati del bacino del Mediterraneo prodotti in Sicilia.

QUALITA' DEI FRUTTI MEDITERRANEI

Mango

Il mango (*Mangífera indica* L.) appartiene alla famiglia delle Anarcardiacee, è nato nella regione Indo-malese e viene prodotto in molte regioni a clima caldo-arido dove si alternano condizioni asciutte, prima e durante il periodo della fioritura, con condizioni di umidità durante la stagione di crescita. In Sicilia alcune località caratterizzate da favorevoli condizioni climatiche sono particolarmente adatte per la sua coltivazione. In particolare nelle province di Catania, Palermo e Messina si coltivano differenti cultivar di mango che coprono un arco temporale di maturazione che va da agosto, con la cultivar precoce "*Glenn*", fino a novembre con la cultivar più tardiva "*Keitt*". Il Mango è composto principalmente da acqua (≥ 80 %) e carboidrati, con un minore contenuto di proteine e grassi. Il carboidrato predominante nel mango acerbo è l'amido che nel frutto maturo viene sostituito in gran parte da zuccheri come saccarosio, glucosio e fruttosio. La buccia e la polpa del Mango sono una fonte di composti bioattivi con potenziale attività salutistica quali carotenoidi, composti fenolici, fibre, acido ascorbico e terpenoidi.

Nell'ambito del presente lavoro di dottorato è stato condotto uno studio su frutti provenienti da un campo commerciale ubicato in provincia di Messina. Sono state prese in esame quattro cultivar di mango ("*Irwin*", "*Glenn*", "*Kensington Pride*" e "*Maya*") e sono stati valutati i parametri qualitativi, nutrizionali e salutistici. I risultati ottenuti hanno confermato il potere salutistico di questo frutto con elevati valori di composti bioattivi quali acido ascorbico, carotenoidi e componenti fenolici.

Ciliegia

Da oltre trent'anni l'industria cerasicola vive in continua evoluzione, con un forte sviluppo di nuovi impianti contraddistinti da una minore dimensione dei fruttiferi grazie a specifiche forme di allevamento e all'uso di nuovi portinnesti. Il Ciliegio (*Prunus avium* L.) è uno dei frutti temperati più popolari, utilizzato commercialmente come frutto da tavola e come ingrediente per cocktail di frutta e ciliegie al maraschino. Le ciliegie sono un'eccellente risorsa di molti nutrienti e sostanze fitochimiche. Esse contengono vari composti fenolici tra cui idrossicinnamati, flavonoli, procianidine e antocianine. Questi componenti sono influenzati da diversi fattori agronomici ed ambientali quali la cultivar, il grado di maturazione, l'ubicazione geografica, la luce e la temperatura. La singolarità e la variabilità delle condizioni pedoclimatiche e agricole del territorio etneo caratterizzano la qualità del frutto influenzando i parametri fisico-chimici e gustativi. La ricerca svolta nell'ambito del corso del dottorato ha valutato parametri fisici e chimici di 24 cultivar di ciliegio coltivate alle pendici del vulcano Etna. Questo studio è stato condotto con l'obiettivo di trovare genotipi promettenti di ciliegio dolce rispetto ai parametri qualitativi e salutistici da impiegare in nuovi programmi di *breeding* per la cerasicoltura siciliana. I risultati hanno dimostrato che le cultivar oggetto di studio presentano un contenuto di antociani e composti fenolici quantitativamente diverso ma il loro profilo qualitativo risulta simile. Inoltre i composti fenolici identificati contribuiscono a determinare la capacità antiossidante totale dei campioni esaminati.

Uva da tavola

La qualità di un frutto è il risultato della combinazione di diversi fattori, non tanto riconducibili alla produzione in campo ma alla gestione post-raccolta e alla trasformazione fino alla tavola del consumatore. Un settore in forte crescita nell'ambito agroalimentare è rappresentato da una

tipologia di alimenti, definita "*ready to use, ready to eat*" ovvero prodotti pronti per il consumo. Prerogativa essenziale di frutti ed ortaggi di IV gamma è quella di evitare al consumatore il lavaggio, la mondatura e il taglio, offrendo così un elevato contenuto di servizio e praticità d'uso. L'uva da tavola minimamente trasformata è stata tradizionalmente utilizzata come componente di macedonie di frutta, e solo di recente è stata presa in considerazione la possibilità di produrre monoporzioni confezionate in piccoli contenitori. L'obiettivo della ricerca, condotta in collaborazione con il Dipartimento di Scienze delle Produzioni Agrarie e Alimentari dell'Università di Catania, è stato quello di valutare la *shelf-life* di piccoli grappoli d'uva da tavola di alcune delle varietà più diffuse in Sicilia quali "*Vittoria*", "*Sugraone*", "*Italia*", "*Crimson*", "*Red Globe*" e "*Perla Nera*" confezionati in contenitori di polietilene. Dopo il confezionamento, i contenitori sono stati conservati a +4° C ed i parametri fisico-chimici e microbiologici sono stati monitorati dopo 0, 7 e 14 giorni di conservazione. Dai risultati ottenuti si osserva che la maggior parte dei parametri chimico-fisici, qualitativi e nutraceutici si sono mantenuti costanti per un periodo piuttosto lungo di conservazione. Inoltre, non è stata osservata alcuna riduzione del potere antiossidante dei prodotti, durante la conservazione.

TRACCIABILITA' DEI FRUTTI MEDITERRANEI

Secondo il regolamento 178/2002 la tracciabilità è "*la possibilità di ricostruire e seguire un alimento, mangime, animale destinato alla produzione alimentare o una sostanza destinata a essere parte di un alimento o di un mangime attraverso tutte le fasi della produzione, della trasformazione e della distribuzione*." Se nel nostro Paese la normativa comunitaria del 2002 in materia di tracciabilità alimentare risulta essere ampiamente applicata (in base a queste disposizioni, ciascun operatore del settore deve essere in

grado di indicare i propri clienti e fornitori e disporre di quei sistemi e procedure che consentano di identificare il prodotto, in modo che ne sia facilitato il ritiro in caso di pericolo per la salute del consumatore), manca un vero impegno verso quella che è stata definita "tracciabilità evoluta", ovvero una vasta gamma di metodologie che puntano al monitoraggio di svariati processi di produzione, al controllo delle tecniche di miscelazione, al trattamento delle materie prime e alla tutela della zona di provenienza. Lo sviluppo di tecniche e metodologie innovative per il controllo dei prodotti alimentari rappresenta una priorità assoluta all'interno dei piani di sviluppo sia Comunitari che Nazionali, per perseguire gli obiettivi di una sempre maggiore sicurezza e tutela della qualità in questo ambito. L'uso di metodologie analitiche non distruttive, rapide per l'applicazione, veloci per la refertazione, efficaci ed altamente performanti rispetto alle matrici indagate, se correttamente applicate per verificare l'autenticità di prodotto, rappresentano un valido ed insostituibile strumento per le autorità preposte ad espletare le funzioni di controllo. Inoltre, l'innovazione scientifica e l'evoluzione tecnologica di strumentazioni e metodologie, possono consentire di individuare rapidamente frodi e adulterazioni particolarmente sofisticate, o specificamente progettate per sfuggire ai controlli di legge correntemente applicati. Un ampio ventaglio di tecniche analitiche e di parametri sono stati studiati per verificare la provenienza dei prodotti alimentari, come l'aroma, il contenuto di zuccheri e il profilo di composti fenolici attraverso metodologie applicate alla cromatografia liquida e gassosa.

La spettroscopia NIR (Near InfraRed), l'analisi multi-elemento e isotopica sono degli utili strumenti analitici di controllo per verificare l'origine geografica di un prodotto alimentare. La spettroscopia NIR si basa sull'assorbimento delle radiazioni elettromagnetiche a lunghezze d'onda comprese tra 780 e 2.500 nm (12.820 e 4.000 cm^{-1}). Le bande di

assorbimento che danno origine allo spettro caratteristico del campione (fingerprint) sono attribuite alle combinazioni di vibrazioni fondamentali relative ai gruppi funzionali presenti nelle biomolecole che compongono l'alimento. Le suddette bande sono molto ampie e sovrapposte, pertanto per processare i dati relativi alle informazioni spettrali è richiesto l'uso della chemiometria. L'analisi multi-elemento si basa su diversi fattori ambientali e geologici, quali il tipo di suolo, le precipitazioni e la temperatura di una regione e fornisce una base scientifica per determinare l'origine geografica di un prodotto. Tuttavia non è solo la presenza o l'assenza di un elemento che è utile ma è la variazione relativa nel profilo dell'oligoelemento il parametro che fornisce il maggiore potere discriminante. I primi studi usavano l'assorbimento atomico per determinare la concentrazione degli elementi. L'introduzione della spettrometria ad emissione ottica accoppiata induttivamente al plasma (ICP-OES) ha permesso di analizzare una più ampia gamma di elementi. La spettrometria di massa di isotopi stabili degli elementi più abbondanti in natura (C, N, O, H, S) costituisce un potente mezzo di indagine per l'identificazione geografica dei prodotti alimentari. I rapporti isotopici di tutti gli elementi presenti in natura subiscono, nel corso dei processi chimici e fisici che caratterizzano l'evoluzione dell'ecosistema terrestre, effetti di frazionamento apprezzabili con le moderne tecniche di misura. Il carbonio utilizzato dalle piante nei processi fotosintetici naturali, deriva interamente dalla CO_2 atmosferica, il quale, dal punto di vista isotopico, è estremamente omogeneo. Durante i processi di fotosintesi si verifica un frazionamento isotopico tra la CO_2 dell'atmosfera e la specie vegetale, che determina, in quest'ultima, un impoverimento dell'isotopo più pesante (^{13}C). Tale frazionamento è funzione del ciclo fotosintetico seguito dalla pianta, nella quasi totalità delle specie riconducibile o al "Ciclo di Calvin" (detto "C3") o al "Ciclo di Hatch-Slack" (detto "C4"). Il contenuto in ^{13}C

caratteristico dei due cicli fotosintetici è molto differente e quindi facilmente distinguibile dal punto di vista isotopico. L'ossigeno e l'idrogeno, così come il carbonio, rappresentano gli elementi più abbondanti nella materia organica. Le variazioni del tenore in ^{18}O e in ^{2}H nelle specie vegetali sono dovute a diversi e articolati processi strettamente correlati a fattori quali temperatura, precipitazioni meteoriche, assorbimento dell'acqua presente nel terreno da parte delle radici, evapotraspirazione, ossigeno dell'atmosfera, fotosintesi, ecc., fattori che a loro volta sono dipendenti dal clima locale.

Con il presente lavoro di dottorato è stato costruito un database rappresentativo e statisticamente valido formato da parametri qualitativi, spettroscopici (NIR) e spettrometrici (ICP-OES e IR-MS) su frutti di agrumi quali arance pigmentate e limoni al fine di sviluppare uno strumento in grado di identificare la loro origine geografica.

Quality and traceability are the winning combination for the development of a modern agriculture, where other functions, related to territorial, environmental and social skills, are gradually taking place aside from the traditional economic task.

In the last decade quality has become an essential feature to ensure the access into the market and an effective competition to many ever-growing exporting countries. Quality is defined as the set of features of a food that leads to consumer satisfaction and includes sensory properties, shelf-life, product safety and functional properties.

The systems for the traceability of food products along specified stages of production, processing and distribution play a key role in assuring safety, quality and origin of food and are typically based on a continuous "paper-trail" and effective labelling. However, analytical techniques that enable the determination of the provenance of a food product provide an

independent mean of control and also help to prove product authenticity, to combat fraudulent practices and to control any adulterations.

The capability to certify food origin or authenticity of a product is of significant economic importance for the agri-food stakeholders in different countries. For example, some food products (organic and/or with certifications of origin) can boast EC recognition on their label. This adds value to such products in terms of marketability and increased export value.

The present work has been developed starting from this perspective, focusing on the quality and traceability of Mediterranean fruits and their derivatives grown in Sicily.

QUALITY OF MEDITERRANEAN FRUITS

Mango

The mango (*Mangifera Indica* L.) belongs to the Anacardiaceae family, is originated in the Indo-Malaysian region, and is produced in many regions characterized by a warm climate where dry conditions before and during the time of flowering alternate with moist conditions during the growing season. In Sicily some localities characterized by favorable climatic conditions are particularly suitable for its growth. Different cultivars of mango are grown in Catania, Palermo and Messina areas where fruits are collected from August ("*Glenn*" is the earliest cultivar) up to late November ("*Keitt*" is the last one). Mango is composed mainly of water (≥80%) and carbohydrates, with a small content of protein and fat. The predominant carbohydrate in the unripe mango is starch, which in the mature fruit is replaced to a great extent by sugars like sucrose, glucose and fructose. Mango peel and pulp are a source of bioactive compounds with potential health-promoting activity, such as ascorbic acid, carotenoids, phenolics, fiber and terpenoids.

In the context of this doctoral work it was conducted a study on fruits from a commercial field located in the province of Messina. Four cultivars of mango have been taken into consideration ("*Irwin*", "*Glenn*", "*Kensington Pride*" and "*Maya*") and quality, nutritional and health parameters were evaluated. The results confirmed the health power of this fruit with high levels of bioactive compounds such as ascorbic acid, carotenoids and phenolic components.

Cherry

For over thirty years the Sicilian sweet *cherry* industry has been evolving, with a strong growth of new plants characterized by a smaller fruiting size thanks to specific types of farming and the use of new rootstocks. Sweet cherry (*Prunus avium* L.) is one of the most popular temperate fruits, utilized commercially as a table fruit and as an ingredient for fruit cocktails and maraschino cherries. Sweet cherries are an excellent source of many nutrients and phytochemicals. They contain various phenolic compounds including hydroxycinnamate, flavonols, procyanidins and anthocyanins. These components are affected by several agronomic and environmental factors such as cultivar, maturity, geographic location, light and temperature. The singularity and the variability of the pedo-climatic and agricultural conditions of the area on the mountainsides of the volcano Etna characterize the quality of the fruit, influencing physico-chemical and sensory parameters. The research, carried out as part of the doctorate course, has evaluated physic-chemical parameters of 24 cherry cultivars grown nearest the volcano Etna. This study has been conducted with the goal of finding new promising genotypes of cherry respect to their qualitative and health characteristics to be used in new breeding programs in Sicily. The results showed that the investigated cultivars had a content of anthocyanins and phenolics quantitatively different but their qualitative profile was similar. Therefore the identified phenolic

compounds contributed to the overall antioxidant capacity of the cherry fruits.

Table grape

The quality of a fruit is the result of the combination of several factors, not only attributable to the field production but also to the post-harvest management and processing until the table of the consumer. A fast-growing sector in food industry is represented by minimally treated products ("*ready to use*" or "*ready to eat*"). Essential requirement for minimally treated fruits and vegetables is to avoid washing, trimming and cutting to the consumer, thus providing a high quality product with ease of use. The minimally processed table grapes have been traditionally used as a component of fruit salads, and only recently it has been taken into account the possibility of producing portions packed in small containers.

The objective of the research, carried out in collaboration with the Department of Agriculture and Food Production Sciences of the University of Catania, was to evaluate the shelf-life of small bunches of grapes for some of the most common varieties grown in Sicily such as "*Vittoria*", "*Sugraone*", "*Italia*", "*Crimson*", "*Red Globe*" and "*Black Pearl*", packed in polyethylene trays. After packaging, the trays were stored at +4 ° C and physic-chemical and microbiological parameters were monitored after 0, 7 and 14 days of storage. Our results showed that most of the physico-chemical and nutraceutical parameters, remained constant over a long period of storage. In addition, no reduction of the antioxidant activity of the products was observed during storage.

TRACEABILITY OF MEDITERRANEAN FRUITS

According to Regulation 178/2002 traceability is defined as "*the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production,*

processing and distribution." If in our country the EC Regulation of 2002 on food traceability is being widely applied (in accordance with these directions, each business operator must be able to show their customers and suppliers and must possess systems and procedures to identify the product, so that the withdrawal is facilitated in case of danger to the consumer's health), there's lack of a true commitment to what has been called "evolved traceability", i.e. a wide range of methodologies that link to the monitoring of several production processes, control of mixing techniques, processing of raw materials and the protection of the area of origin. The development of innovative techniques and methods for the control of food products is a top priority in both National and European development plans, to pursue the objectives of increasing safety and protection of food quality. The use of non-destructive analytical methods, rapid for their application, fast for reporting, effective and high performing with respect to the investigated matrices, if properly applied to verify the authenticity of products, represents a valuable tool for the authorities in charge of control functions. In addition, scientific innovation and technological evolution of tools and methodologies, can be helpful to quickly identify sophisticated adulteration or frauds specifically designed to evade inspections. Many analytical techniques and parameters have been studied to verify the provenance of foods, such as aroma, sugars and phenolic compound profiling, by methodologies applied to the liquid and gas chromatography.

NIR (Near InfraRed) spectroscopy, multi-element and isotopic analysis are useful analytical tools to verify the geographic origin of a food product. NIR spectroscopy is based on the absorption of electromagnetic radiations at wavelengths ranging between 780 and 2500 nm (12820 and 4000 cm^{-1}). The absorption bands that give rise to the characteristic spectrum of the sample (fingerprint) are attributed to the combinations of the fundamental

vibrations relating to functional groups present in the food biomolecules. The above-mentioned bands are very wide and overlapping, therefore to process data relating to the spectral information the use of chemometrics is required. The multi-element analysis is based on various environmental and geological factors, such as soil type, rainfall and temperature and provides a scientific basis for determining the geographical origin of a product. However it is not only the presence or absence of an element that is useful but the relative change in the profile of that element is the parameter that provides the greatest discriminating power. Early investigations used atomic absorption to determine elemental concentrations. The introduction of inductively-coupled plasma-optical emission spectrometry (ICP-OES) allowed a wider range of elements to be analysed. Mass spectrometry of stable isotopes of the most abundant elements in nature (C, N, O, H, S) is a powerful mean of investigation for the geographical identification of food products. The isotope ratios of all the elements in nature undergo, due to chemical and physical processes that characterize the evolution of the terrestrial ecosystem, fractionations detectable with modern techniques. The carbon used by plants in natural photosynthetic processes, derives entirely from atmospheric CO_2 which has a extremely homogeneous isotopic composition. During the process of photosynthesis isotope fractionation occurs between atmospheric CO_2 and plant species, determining, in the latter, a depletion of the heavier isotope (^{13}C). This fractionation is function of the photosynthetic cycle followed by the plant, in almost all of the species due to the "Calvin cycle" (called "C3") or the "Cycle of Hatch-Slack" (called "C4"). The content in ^{13}C characteristic of the two photosynthetic cycles is very different, and therefore easily distinguishable respect to the isotope composition. The oxygen and hydrogen, as well as the carbon, represent the most abundant elements in the organic matter. Changes in the content of ^{18}O and ^{2}H in plant species

are due to different and articulated processes and are closely related to factors such as temperature, rainfall, water absorption from the soil by the roots, evapotranspiration, atmospheric oxygen, photosynthesis, etc., factors that are dependent on the local climate.

With this doctoral work it was built a representative and statistically valid database consisted of qualitative, spectroscopic (NIR) and spectrometric (ICP-OES, IR-MS) parameters of citrus fruits such as blood oranges and lemons, in order to develop a integrated tool able to authenticate their geographical origin.

QUALITY AND TRACEABILITY OF TYPICAL MEDITERRANEAN FRUITS

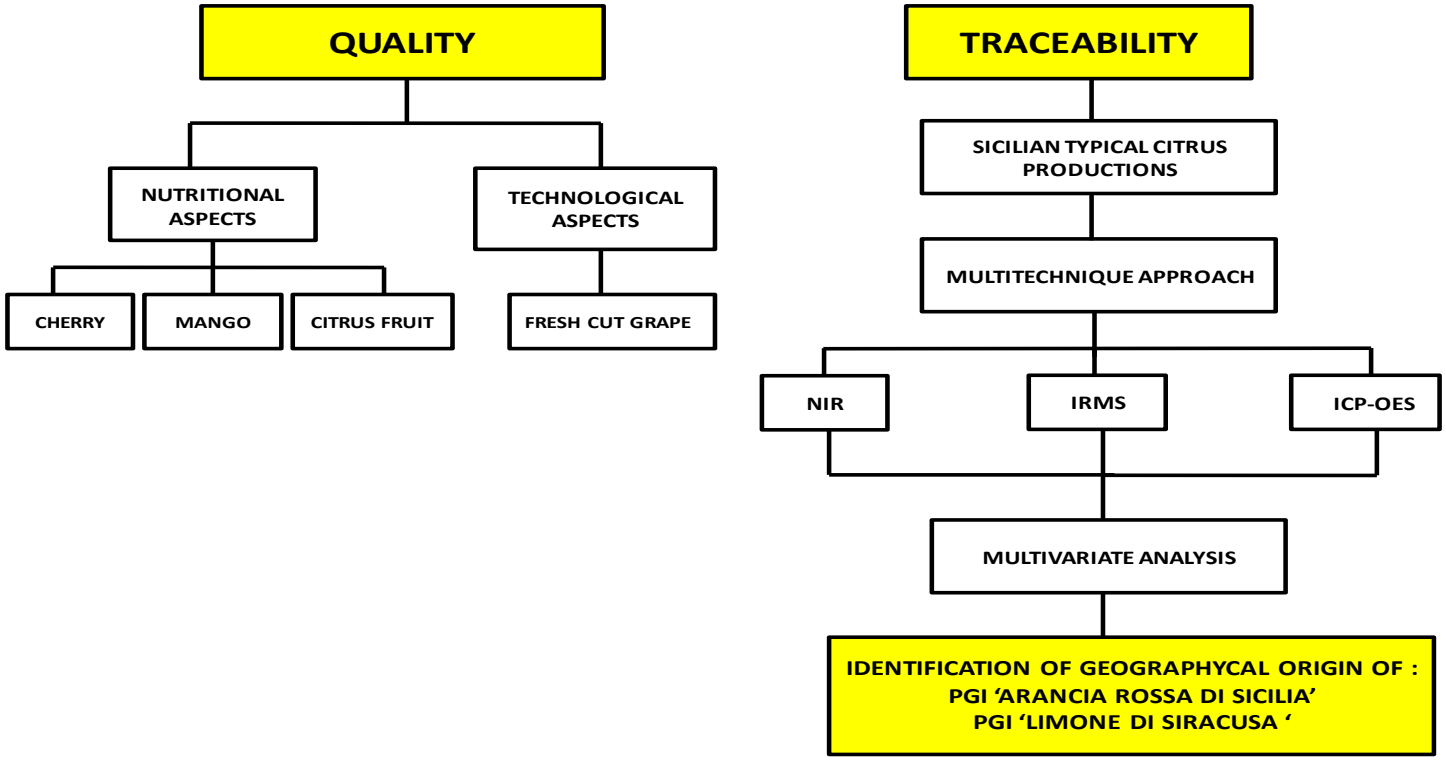


FIG. 1 EXPERIMENTAL RESEARCH PLAN

2. State of the art

2.1 QUALITY OF MEDITERRANEAN FRUITS: NUTRITIONAL AND TECHNOLOGICAL ASPECTS

"Food quality" is a very wide issue and sometimes it is also difficult to define. Food is generally a complex mixture of chemical compounds and physical properties which make up its characteristics.

The quality of food is commonly defined as "the set of features of a product able to satisfy the consumer needs and determine its value " (1).

Up to the years preceding the second world war the agri-food market offered a good choice of products, present in high quantities. It was usual a family production of several products, arising from orchards, gardens and stables, intended for self-consumption or for a small sale.

There was no industrial production but many local producers. The storage of food was limited in time because they lacked the technical knowledge that would be gained in the following decades.

In the mid 1950s occurs the so-called economic boom, including the agricultural sector. Food consumption change as composition, thus adopting more productive farming techniques based on the use of improved varieties, of pesticides and on the mechanization. Begins food processing industry and a network of distribution channels.

In the early 1970s the West faces a serious oil crisis causing a rising prices accompanied by a stability in consumption and a differentiation thereof.

Because of this, companies turned to new types of products and began to believe a element, which until then had been little or not at all considered: the consumer.

The consumer becomes the economic entity more involved in the use quality and the satisfaction of his requests represents the essential aspect in a production that from "*market oriented*" became "*consumer oriented*".

The quality differs from individual to individual and depends on the points of view of the consumer. Kapsak et al (2) stated that "the quality depends on the person, place and time".

The sequence of consumers motivation to purchase can be expressed as follows:

Therefore, from the consumer's point of view it is necessary to differentiate various types of quality:

-*Expected Quality* is the set of features that customers expect to find in the product in order to see fulfilled its needs and expectations, implied and expressed.

-*Promise Quality* are consumer expectations induced by a particular brand or trademark, based on a prior act of consumption or experience. What differentiates the quality promised by a product to another are communication elements such as design, the appeal of the brand image, price, point of sale, advertising, etc.

-*Perceived Quality* is the summation of promise and actual quality is the perceived quality, to which are added the product and communication.

The relationship between perceived quality and expected quality determines customer satisfaction.

SATISFACTION = PERCEIVED QUALITY/EXPECTED QUALITY

In the past, "food quality" has been identified with the nutritional and sensory properties of foods; in the last decade there has been an extension and a enrichment of the concept of food quality.

AGRONOMIC QUALITY:

It includes the characteristics sought by farmer relate to the breeding and cultivation mode: disease resistance, high productivity, adaptability to production environment (climate, soil). This kind of quality impacts heavily on the choice of crops or livestock.

SENSORY QUALITY:

The sensory quality of a food depends some characteristics of the food itself, such as its appearance, aroma and texture, perceived through the sense organs. It is therefore subject to personal assessments of consumers who are strongly influenced by psychological factors, social and cultural.

PRODUCT QUALITY:

The factors that make a product more enjoyable in the phase distribution are the shelf life, the packaging and responsiveness to specific needs of the market. The shelf life is a particularly

important feature, because it influences the duration of product on the market: some foods are inherently preserved for long enough periods, while others, as with very active metabolism, can be kept fresh for only short periods.

The package is not only intended as means for containing a food and protect it from contamination but also has the function of marketing becoming thereby strategic tool in hand to the company of production to stimulate the purchase by the consumer.

HEALTH QUALITY

The health quality is represented mainly by the absence of pathogenic microorganisms or any substance that can affect the health of the consumers.

Contamination may be:

• Chemical is determined by pesticide residues, heavy metals, mineral oil, hand sanitizers (detergents and disinfectants)

• Biological is caused by the presence and proliferation of pathogenic bacteria, mycotoxins, viruses;

• Physical is caused by presence of foreign matter, pH, temperature, Aw different from optimal conditions.

TECHNOLOGICAL QUALITY:

The technological quality of a food is the set of characteristics that makes it suitable to processing, or to a particular technological intervention in relation to the final product to be obtained. The technological quality will differ depending on the type of product to be processed and, for the same product, the kind of transformation.

Minimal processing technologies have made it possible to obtain and provide food for consumers that are resized the negative consequences arising from traditional technologies, such as the reduction of nutrients and alteration of the organoleptic characteristics. This is due to the use of transformative treatments and minimized stabilization (low intensity) and avoiding the use of additives of synthetic preservatives. Fruits and vegetables are subjected to technological intervention either in working phase during the operations of sorting, washing and cutting and during stabilization and packaging. Usually indicated by the term 'fresh-cut products', these foods are part of a broader category, defined as "*ready to use*" or "*ready to eat*", packaged products and direct consumption, requiring no previous operation.

Qualitative factors offered are in fact comparable to those of the fresh product: high nutritional value, organoleptic quality and therefore a welcome image of freshness and genuineness. Essential prerogative of these fruits or vegetables processed is to avoid the consumer washing, cleaning and cutting, thus offering a high service content and ease of use. Fresh-cut products, however, are generally a shelf-life (life expectancy) limited to a few days, determined by combining multiple stability factors. Preliminary operations to which the raw materials are, in fact, cause some damage physiological and mechanical induction managers and/or acceleration of enzymatic and chemical reactions. Following these reactions, occurring in products partially phenomena such as unwanted loss of tissue consistency, enzymatic browning and, more generally, degradation of pigments (for example, chlorophyll

and anthocyanins), microbial attack favored by percolation of fluids, cellular oxidation processes due to the presence of oxygen inside the product and/or in the environment in which it is located and accelerated reactions from the influence of external factors.

In Italy "*ready to eat vegetables*" appeared five years after France, in the 80's when was recorded the rise of the families *dual career* who urged the change of food culture linked to the consumption of fresh bulk.

The companies that transformed these products in Italy were about thirty, of which 20 located in Lombardia. The level of concentration was very high, with two or three dominant companies. The South was marginal compared to the transformation because it had only four companies, but provided a high percentage of vegetables to be transformed, mainly from Campania and Puglia.

Today the minimally processed have gained a large market share.

The assortment of products "*fresh cut*" consists of: peeled potatoes and sliced, also ready for cooking in the microwave, cut lettuce and cabbage, spinach washed into individual portions and family, slices of fruit packed in containers such as peaches, mango, melon and moreover equipped with a spoon for tasting, snack of carrots and celery cut into julienne and salads dipped in sauces, diced onion, bags of vegetables prepared with fresh sauces for dressing trays of vegetables.

NUTRITIONAL QUALITY

The term "nutritional quality" indicates primarily the nutritional value of the food in terms of balanced intake of carbohydrates, fats, proteins, minerals and vitamins essential for health and well-being.

The nutritional value consists of the following two aspects:

- quantity, how many calories provides per unit weight;
- quality, expressed in terms of composition of nutrients, i.e., percentages of carbohydrates, proteins, lipids, vitamins, minerals, fiber, etc.

Of each nutrient should be considered to its bioavailability according it is easily assimilated into the food or chemical transformation requires the body to become assimilated, or its assimilation is hindered by the presence of anti-nutritional factors.

Fruits and vegetables are also an excellent source of phytochemicals in addition to contributing to a healthy diet. They contain various phenolic compounds including hydroxycinnamate, flavonols, procyanidins and anthocyanins. These components are affected by several factors such as cultivar, maturity geographic location and environmental factors such as light, temperature and various stresses.

All these bioactive compounds are good antioxidants and their daily intake in the diet has been related to prevention of degenerative processes, such as cancer and cardio and cerebro-vascular diseases (3,4)

2.1.1 Mango

The mango (*Mangifera Indica* L.) belongs to the *Anacardiaceae* family, originated in the Indo-Malaysian region, described as the most favored and valuable fruit through-out the tropics is of the major economic concern. The mango is the produced in many regions of warm climate where dry conditions before and during the time of flowering alternate with moist conditions during the growing season.

Mango has been cultivated for about 4,000 years and its production and consumption has gradually increased as its popularity has grown. Originating over 4,000 years ago in India and Burma, its cultivation has spread to Malaysia, Eastern Asia and Eastern Africa. Mango production is highest in India, at 41% of the world's production (10,800,000 MT), followed by China, Thailand, Mexico, Pakistan, Indonesia, the Philippines, Nigeria and Brazil (FAO, 2004); Mexico is known at the leading mango-exporting country (41% of the world market, 102,500 MT), followed by the Philippines (7,8%) and Pakistan (7,6%) (5)

The world's largest mango-importing country is the US has steadily grown in response to increasing domestic demand.
Mango are well adapted to tropical and sub-tropical regions of the word but production in the United States (US) is comparatively small, due to climatic limitations. Florida is the only state in the United States where agricultural statistics are reported for mangoes, even through mangoes are also cultivated to various extents in Hawaii, California, Texas and Puerto Rico (6).
In Sicily some areas characterized by favorable climatic conditions are particularly suitable for its growth. In Catania, Palermo and Messina areas different cultivars of mango are grown and fruits are collected from August ('Glenn' is the earliest cultivar) up to late November ('Keitt' is the last one).
Most of word's mango production is consumed raw as a dessert fruit, the rest of it being processed into diverse products such as nectar, juice powder, canned mango slices in syrup, chutney, etc.

Mango is composed mainly of water (≥80%) and carbohydrates, with a small content of protein and fat. The predominant carbohydrate in the unripe mango is starch, which in the mature fruit is replaced to a great extent by sugars like sucrose, glucose and fructose.
Ali (7) reported that the free sugars in the mango fruits mainly consist of glucose, sucrose, while xylose and arabinose have also been detected in similar quantities during different stages of fruit ripening. However, it has been reported that there is an increase in reducing sugars and total soluble solids, during storage period. The acidity of the fruit expressed in term of

citric or malic acid, since they are the main accumulated free organic acids contributing to the acidity of the fruit besides tartaric, oxalic and glycolic (8).

The external color of the fruit is an important factor in consumers´ preference. The only significant external color changes during ripening are the disappearance of chlorophyll, the apparent increase in anthocyanin pigments and significant increase of the total carotenoids and β-carotene in the peel and pulps (9).

Mango peel and pulp are a source of bioactive compounds with potential health-promoting activity, such as ascorbic and dehydroascorbic acids, carotenoids, phenolics compounds, fiber, terpenoids. Gallic acid and gallotannins were the first compounds defined as major polyphenolics present in mango (10) with other polyphenols, such as mangiferin, quercetin, kaempferol, p-OH- benzoic acid, *m*-coumaric acid, *p*-coumaric acid and ferulic acid. These phenols were found to naturally decrease during storage, due to ripening, resulting in loss of astringency, which is a characteristic of mango.

There are over 25 different carotenoids that have been separated from mango pulp and the most abundant is beta-carotene which is responsible for the yellow-orange pigmentation of the majority of mango varieties.

Mango is also rich in vitamin C. Vitamin C is involved in the formation of red blood cells, collagen, bones and teeth and enhances the absorption of iron in food, while strengthening the body's defense system against infections and allergies, reduce cholesterol levels and slows the aging process of cells.

All these bioactive compounds are good antioxidants and their daily intake in the diet has been related to prevention of degenerative processes, such as cardiovascular diseases and cancer.

2.1.2 Sweet cherry

Sweet cherry (*Prunus avium* L.) is one of the most popular temperate fruits. This fruit is important commercially as a table fruit and as an ingredient for fruit cocktails and maraschino cherries.

According to the FAO Statistical Database (FAO, 2010), the world production of sweet cherries is 2,102,651 t. The top worldwide producer is Turkey (417,905 t), followed by the United States of America (287,305 t), and Iran (255,500 t). Producing about 6% of the cherries in the world (115,476 t), Italy is the fourth largest producer (FAO, 2010). Sweet cherries have high commercial importance in Sicily, where there has been strong development over the last thirty years due to the introduction of new varieties and new orchards.

The singularity and the variability of the pedo-climatic conditions of the areas nearest to the Etna volcano characterize the quality of cherry fruit, giving it intrinsic sensory parameters.

The hills where the cultivation of cherries are being develop range between 400 and 600 meters, but productive appearances are not rare around 1200 meters.

CHERRY VARIETIES

According to the peculiar characteristics of pulp, the cherries are divided into:

Lustrine, aquaiole o tenerine (fresh or soft pulp) :

1. *"Maiolina precoce o virifica"*

Elongated fruit, weight of 3 g, with skin and pulp very juicy, slightly acidic, soft and low shelf-life. Blooms in mid-March and the first fruits ripen from the third decade in April, until the first two weeks in May;

2. *"Maiolina a rappu"*

The fruit is similar in color to the "*Maiolina precoce*" but more rounded, with slightly soft and acidulous pulp. For the characteristics of earliness of ripening, disease resistance and high productivity met local farmers interest.

3. *"Toscana"*

Roundish fruit weighing 4-5 grams, with red tender flesh-grenade and sweet enough. Due to its poor keeping and to be receptive to diversity parasitic diseases, is not very widespread;

4. *"Maredda"*

Fruit weighing 3-4 gr., conical, reddish rind first and glossy black, to complete ripening; flesh juicy, tender and slightly sugary. The fruits are of mediocre flavor.

Semiduracine (medium consistency pulp)

1. *"Napoleona"*

Spherical fruit, garnet-black peel, medium consistency and enough pulp, pleasant flavor. Large size 6-7 gr, good keeping qualities and great appreciation. The fruits ripen in the second decade of June;

2. "*Minnulara*"

Fruit 4-5 weight gr., spherical, with velvety-black skin, red pulp, good texture, very sweet, pleasant and quite storability;
The fruits ripen in June.

3. "*Raffiuna*"

Spherical fruit, heart shaped, weighs 7 g. and presents high shelf life. Black-rind peel, velvety red flesh with white marbling formed by fibro-vascular bundles, enough juicy and refreshing.

Duracine (crispy pulp)

1. "*Napoletana*"

Red pulp, compact and quite sweet fruit, discreet commercial appreciation. The fruits ripen in June.

In relation to ripening :

Early varieties:

1. "*Sweet Early*"

High vigor with medium-sized flowering, fruit is self-fertile, coarse, slightly sour.

Medium-early varieties:

1. "*Early Star*"

High vigor with media entities flowering. The fruit has good size, texture and intense color;

2. "*Grace Star*"

Medium - high vigor and late bloomers. The ripening is uniform, with good grip. It is a very productive varieties, fruit quality is characterized by big size.

Middle varieties:

1. "*Giorgia*"

Fruit with very good firmness and good productivity. It has dark red flesh and skin. The flavor was a good sweet-acidic flavor;

2. "*Black Star*"

High productivity and quality, great size, bright color, good texture and flavor.

Medium-late varieties:

1. "*Ferrovia*"

The most representative varieties. It is very attractive, has large fruits and excellent taste. This heart-shaped, bright red cherry is particularly appreciated for its juicy, firm and sweet pulp.

Late varieties:

1. "*Sweetheart*

A large, bright red cherry. Fruits of good size and excellent quality. Has a mild, sweet flavor and outstanding firmness. This heart-shaped cherry ships extremely well, and will keep your late July and August cherry displays full.

QUALITATIVE ASPECTS

Sweet cherries are an excellent source of many nutrients and phytochemicals in addition to contributing to a healthy diet. They contain various phenolic compounds including hydroxycinnamate, flavonols, procyanidins and anthocyanins (11, 12). These components are affected by several factors such as cultivar, maturity geographic location and environmental factors such as light, temperature and various stresses.

The major polyphenols in cherries are anthocyanins and hydroxycinnamic esters, secondary metabolites evolved by plants as a natural defense system.

The major anthocyanins identified in cherry fruits were the 3-*O*-glucoside and 3-*O*-rutinoside of cyanidin, with peonidin-3-*O*-rutinoside as well as pelargonidin-3-*O*-rutinoside being present in much lower amounts (13, 14, 15) and these components are important for their potential contribution to the color of the cherry fruits (16, 17).

Color is the most important indicator of maturity and quality for both fresh and processed cherries.

Sweet cherries are also rich in phenolic acids such as hydroxycinnamic acid derivatives (neochlorogenic acid, *p*-coumaroylquinic acid and chlorogenic acid) (18; 13). Moreover, strong correlations were found between the phenolic content and antioxidant activity of fruits (19; 20)

It has been demonstrated that the consumption of sweet or sour cherries reduces the risk of cancer (21) as well as pain from arthritis and inflammation (21, 22, 23) and offers protection against neurodegenerative diseases (24).

2.1.3 Table Grape

The fruit of the grape is a berry. Berries are attached to the stem. Many berries make up the cluster or bunch of grapes. The essential parts of the berry include the skin, pulp, and seeds. The skin consists of an outer layer covering the berry. It is made up of six to ten layers of thick walled cells. The outer surface of the skin is covered with a wax-like coating called the cuticle, which renders the berry waterproof. The main components in the skin are: coloring matter (red and/or yellow pigments), tannins, aromatic substances, potassium and other minerals. Below the skin layer lies flesh or pulp which makes up most of the berry volume. Cells in the pulp have large vacuoles containing the juice. When the berry is gently crushed, the fragile cells in the pulp are broken and the juice is released. This juice is commonly referred to as the free run.

The seeds are localized in the center of the flesh. The berry contains two to four seeds. They are rich in tannin which is extracted during fermentation (in red wines).

Freshly expressed grape juice consists of 70 to 80% water and many dissolved solids.

These soluble solids include numerous organic and inorganic compounds. In grapes, a large portion of the soluble solid is sugars. Glucose and fructose are the main sugars in the juice. The sugar content of the juice of ripe grapes varies between 150 to 250 g/L. In unripe berries, glucose is the predominant sugar. At the ripening stage, glucose and fructose are usually present in equal amounts (1:1 ratio). In overripe grapes, the concentration of fructose exceeds that of glucose. In ripe grapes, there is some variation in the glucose to fructose ratio among the grape varieties.

Next to sugars, organic acids are the most abundant soluble solids present in grape juice, responsible for the tart taste. The principal organic acids found in grapes are tartaric, malic, and to a small extent, citric. Many other organic acids, including amino acids, are also found in juice and wines, but tartaric and malic acid account for over 90% of the total acids present. During the early period of berry growth, concentration of both acids increases in the fruit. With the onset of ripening, as the sugar accumulates in the fruit, the acid concentration decreases. Generally the reduction in malic acid is greater, and consequently, at maturity, the fruit contains more tartaric acid than malic. Grapes are one of the rare fruits that contain tartaric acid. It is present as free acid and a salt, such as potassium bitartrate, an important constituent which affects pH and the cold stability of the wine.

The acid composition of grapes is influenced by many factors such as variety, climatic region, and cultural practices.

Generally in ripe grapes, the acid levels are lower in a warmer climatic region than in a cooler region.

Phenolic compounds are important constituents of grapes. They play a vital role in determining the market value of table grapes and also the quality of red wine and juice. These substances are primarily located in the seeds and skins of the berry and the juice contains a very small amount (3 to 5% of total phenols). These compounds show antioxidant and anticarcinogenic properties and are supposed to be mainly responsible for preventing cardiovascular disease (25,26), cancer (27,28) and other degenerative disorders such as Alzheimer's disease or dementia (29), especially through moderate daily consumption of red wine during a long period of time. The two main substances included in this group of compounds are anthocyanins and tannins.

Anthocyanins are responsible for the red and purple color of the grapes.

Tannins are very complex compounds. They are large molecules with a molecular weight over 500. They are yellow, brown, and red colored. They are astringent and bitter. During processing and aging, the tannins polymerize and the polymerization leads to increased molecular size.

According to the Food and Agriculture Organization (FAO), 7,272,583 ha of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit (table grape), and 2% as dried fruit (raisin). However, an important quantity of table grape is lost at various points between harvest and consumption.

Table grape industry in Italy is currently involved in a process of economic contraction due to the change of the world scenario: there is a significant regression of the European productions in comparison to those coming from Asia and other emergent countries. This dynamism has strongly conditioned the Italian productions that find several difficulties in the international markets and are less remunerate in comparison to the foreign ones. Due to this dynamism it is really important to find innovations that can support the competitiveness of the productions within the Italian and foreign markets. An important impulse to the commercial diversification is driven from the consumers, that require a high-level qualitative product regarding nutritional, sensorial and hygienic-sanitary profile; moreover today, due to life-style and alimentary habits, there is an increasing request of products ready for the consumption and with a sustainable cost.

The possibility to introduce innovation such as fresh-cut table grapes represents an opportunity for table grape growers and consumers. Consumers are very interested in products "*ready to eat*" and "*read to use*".

Freshness, nutritional content and sensorial aspects like visual quality and taste represent the main important characteristics.

The quality of fresh-cut grape can be affected by intrinsic factors, such as the morphology, physiology, biochemical defense mechanisms, genotype, ripening stage upon processing, etc., and extrinsic factors, such as temperature, humidity, quality of the cutting phase, chemical treatments, etc. These factors influence a number of changes, which meets the

grape after processing, such as loss of color, reduction of ascorbic acid content, the formation of bad odors, softening and loss of texture and tissue development of the microbial load.

To keep table grapes fresh and increase shelf life, scientists are seeking advanced techniques that provide healthy, safe alternatives to conventional packing methods.

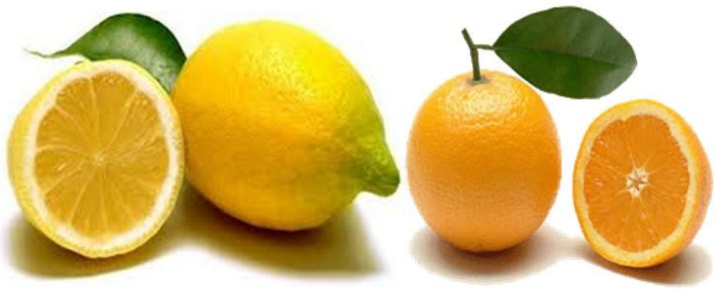
Modified atmosphere packaging (MAP) is the most common manner to preserve the initial color of fresh-cut fruits (30). Modified atmosphere (MA) conditions can be done via packaging, which is a passive system, by balancing produced respiration and gas exchange through package materials. There has been an increase in the use of plastic film packaging, such as low density polyethylene (LDPE), polyvinychloride (PVC) and polypropylene (PP) for fresh fruits and vegetables (31). These materials are generally transparent, provide a barrier to water vapor.

Yaguang Luo and William Conway from the USDS ARS, in collaboration with Liping Kou, Wu Ding, and Xinghua Liu from China's Northwest A&F University, recently published a report in HortScience that explored alternatives to sulfur dioxide for maintaining quality of table grapes.

However there are disadvantages to sulfur dioxide use. The concentration of SO_2 necessary to inhibit fungal growth may induce injuries in grape fruits and stems, and sulfite residues pose a health risk for some individuals. Applications of SO_2 have been restricted in many countries, making it essential to identify safe, alternative technologies that effectively control fungal growth and assure high-quality fruit.

Kou et al. (32), concluded that "the combination of treatments such as low temperatures, reduction of the amount of oxygen and use of citric and ascorbic acid as anti-browning agents, maintained excellent visual quality preventing dehydration, and delaying softening and senescence of grapes".

2.1.4 Citrus fruit



There is no doubt that the original genetic pool of the citrus plants originated in South-Eastern Asia. According to R.W. Scora, *Citrus maxima* (*C. grandis*), pummelo, *Citrus medica*, citron, *Citrus reticulate*, mandarin and similar, *Citrus halimii*, a recently discovered taxon, are the parent species of all the citrus known today. *Citrus grandis* was probably the first ancestor. It originated in Malaysia and Malay archipelago. *Citrus medica* originated in India and *Citrus reticulata* in China, *Citrus halimii* in Thailand and Malaya. All other species derive from cross-pollination between these biotypes:

- The common orange (*Citrus sinensis*) and the sour orange (*Citrus aurantium*) are considered hybrids of the pummelo with some mandarin.
- Limes (*Citrus aurantifolia*) and lemon (*Citrus limon*) originated through the hybridization of citron with some primitive Papeda (wild species from Asia of no commercial value).
- The kumquat (Fortunella spp) comes from mandarin.
- The grapefruit (*Citrus paradisi*) is a hybrid of pummelo.

Citrus fruit are modified berries known as hesperidia. Characteristics of this type of fruit are the

following: fruits are more or less spherical although depending on the species can be flattened like mandarins or rather ellipsoid like lemons or limes. The external part of the rind is called flavedo; it is rather thin, with a pigmentation that varies according to the species or variety, with colours from deep orange or reddish to light orange, yellow or greenish. Also in the flavedo are numerous essential oil glands, spherical in shape and of different sizes, that can be prominent as in pummelo, and sweet orange or grapefruit, or sunken as in sour orange or mandarin.

The internal part of the rind is the albedo, name that comes from latin (albus white), has an ivory or pale yellow colour and is spongy in texture. The thickness and consistence of the albedo varies with the species.

Mandarins have a very thin albedo, medium to thick in grapefruits, and very thick in shaddocks and citrons. Both flavedo and albedo form the rind, that is usually the non edible part of the fruit.

The edible part of the fruit is integrated by the segments or locules. The cross section of the fruit shows the form of each locule, frequently around 8-12 in most citrus, like corresponds to the number of carpels that were present in the ovary. Each locule is covered by a membrane, slightly coarse and covered by vascular bundles that transfer nutrients for growing of the fruit. Inside the membranes are the juice vesicles in a high number, each containing the juice within the vacuole of the cell. The central axis of the fruit is called the axis or columnella, that has a consistence and texture very similar to the albedo. In some cases, a modification of the fruit occurs that is called the

navelisation or formation of the navel that is like a secondary smaller fruit, developed at the upper part of the main fruit. When navel is present it can be visible from the outside, and can be observed in detail when the fruit is peeled.

Mature fruits have the calyx present at their base, while at the stylar end usually is only present a small scar where the style was fused. Around this scar an areole may exist, consisting in a more or less depressed and rather smooth rounded area. The shape of the fruit varies with the species, being either spheroid, ellipsoid, piriform, oblique, oblate, ovoid-oblique or ovoid. The base of the fruit may have the following shapes: necked, convex, truncate, concave, concave collared or collared with neck. The apex of the fruit can be: mammiform, angular, convex truncate or depressed.

2.1.4.1 *Sweet oranges*

From the agronomic viewpoint, Sweet Oranges belong to the species *Citrus sinensis* (L.) Osbeck, being the main citrus tree grown in most of the citrus producing countries. Four groups exist within this species:

1. Navel oranges: can be distinguished by the presence of a navel at the stylar end of the fruit. They include cultivars like "Washington", "Thompson", "Navelina", "Navelate" and "Newhall".

2. Common oranges: also called blond oranges. They include most of the older varieties grown in different countries, having local names and being difficult to distinguish. Some known cultivars are: "Cadenera" from Spain, "Jaffa" from Israel,

"Biondo Comune" from Italy, "Pera" from Brazil, or "Pineapple" and "Valencia" from USA.

3. Acidless oranges: also called sugar or sugary oranges because of the low acidity of the juice. Some cultivars are "Vaniglia", "Sucreña" or "Imperial" and "Succari" (33)

4. Blood oranges: characterized by the presence of anthocyanin in the fruit, giving it a more or less intense red color to the juice, the pulp, or the rind. The main cultivars of this group are "Moro", "Tarocco" and "Sanguinello".

TAROCCO

Shape: globose or obovata based more or less prominent ("muzzle" long or short);

Skin color: orange with red colored parts more or less intense garnet;

Pulp color: orange with red streaks more or less intense depending on the time of harvesting;

Minimum gauge: 10 (diam. mm. 60/68);

Yield: 40% minimum juice;

Total soluble solids content in the juice: minimum Brix 10.0;

Maturation ratio: 7.0 minimum, determined as Brix/acid ratio, expressing the acids as citric acid anhydrous.

- **MORO**

Shape: globose or ovoid;
Skin color: orange with more intense shades on one side of the fruit;
Pulp colour: red wine maturing entirely advanced;
Minimum gauge: 10 (diam. mm. 60/68);
Yield: 40% minimum juice;
Total soluble solids content in the juice: minimum 10, expressed in degrees Brix;
Maturation ratio: 6.5 min, determined as Brix /acid ratio, expressing the acids as citric acid anhydrous. Can be tolerated the ratio of 5.5 for the fruits collected in December.

-SANGUINELLO

Shape: globose or obovata;

Skin color: orange with red nuances;

Pulp color: orange with red streaks;

Minimum gauge: 10 (diam. mm. 60/68);

Yield: 40% minimum juice;

Total soluble solids content in the juice: minimum Brix 10.0;

Maturation ratio: 8.0 minimum determined as Brix/acids ratio, expressing the acids as citric acid anhydrous.

In these cultivar, sugars and organic acids can be considered the core components of orange juice. The sugar content contributes substantially to the formation of values of the "Ratio" defined as the ratio between the total soluble solids and acidity expressed as a percentage of citric acid. The value of the "Ratio" increases with the progress seasonal and is indicative of the degree of ripeness of the fruit. Juices from different species, non-reducing sugars (sucrose) and reducing

(glucose and fructose) are present in different ratios. In ripe oranges, three sugars are present, in order, in the ratio 2:1:1.

Organic acids are among the principal soluble constituents of the juice. Citric acid and malic acid are present mostly free and partly combined mainly with potassium which represents about 60-70% of total cations in juice. It follows that the orange juices are veritable buffer systems and as such, are subject to only is minor changes in pH during the various stages of ripening of the fruit. In orange juice were identified numerous other organic acids, including isocitric, oxalic, succinic, acetic and formic. Ratio citric/isocitric and citric/malic are of particular analytical interest for the purpose of establishing authenticity of fruit juices.

Blood orange contains vitamin C and other bioactive compounds, including flavonoids and hydroxycinnamic acids, with potential health-promoting properties (34).

Vitamin C is the most important of all vitamins and its properties have been extensively proven by numerous scientific studies: is a strong antioxidant and destroy oxygen free radicals (35), participates in the processes of cellular respiration, intervenes in the synthesis of collagen (36). Ascorbic acid is the most potent activator in the absorption of iron (37, 38) and increases the bioavailability of iron in all products fortified with this element (39).

Flavanone glycosides are the most abundant phenolic compounds present in citrus fruit, but significant concentrations of other flavonoids such as metoxylated flavones, flavonols, and anthocyanins (the last only in blood

oranges) have also been found (40). Previous studies have shown that hesperidin, the principal flavanone, has antioxidant (41),antitumor (42), and anti-inflammatory (43) properties.

Hydroxycinnamic acids possess significant antioxidant activity and chemoprotective effects, as shown by *in vitro* and *in vivo* studies (44, 45).

Ferulic acid is a major component of hydroxycinnamic acids, followed by p-coumaric, caffeic and sinapic and the total concentration of all four hydroxycinnamic acids increases with maturation progresses.

Anthocyanins are glycosylated derivatives of salts of flavilio polyhydroxylated and/or polymethoxylated, which originate from flavanones contained in the albedo and in the membranes through a reduction reaction catalyzed by specific enzymes (46) and accumulate in the aqueous vesicles endocarp being soluble in water.

Previous studies have demonstrated that the major anthocyanins in blood orange juice are cyanidin-3-glucoside (Cy3G) and cyanidin-3-(6″malonyl)-glucoside (Cy3MG) (47), and that their level in the fruit always varies according to the following order: Moro>Sanguinello>Tarocco. Moreover, marked differences in anthocyanin content among individual Tarocco clones were observed (48).

They have also been associated with potentially beneficial effects against various diseases such as capillary fragility, diabetic retinopathy, and human platelet aggregation (49). In addition, anthocyanins are known to be potent antioxidants (50, 51), and anthocyanin-rich fruit or juice has been associated with higher antioxidant activity.

Orange juice is also an excellent source of potassium and folic acid (Vitamin B9), which is recommended for women who are pregnant or may become pregnant, and a valuable source of iron, calcium and vitamin A.

2.1.4.2 *Lemon*

The true origin of the lemon is unknown, although some think that derives from north-western India. Arabs distributed it widely in the Mediterranean region between 1000 and 1150 A.D.

Lemon is a thorny tree that, depending on the variety, can be represented by specimens small, medium or large. Canopy is generally open, buds with purplish apical section, leaves elliptic, acuminate, of green colour. The flowers can be male or hermaphrodite, because of abortion more or less complete of the harem, the petals are fragrant, purplish color on the outside and white on the inside.

The fruit, said hesperidium, can take different forms, spherical, oval, elliptical, with more or less thick skin from the typical lemon yellow when ripe. Seeds are white and small, oval, pointed, smooth on the outside and contain more embryos.

Differs from other cultivars for production of new green shoots, instead of the usual violet color and white flowers very similar to those of the orange.

Lemon is a typical re-flowering species; the same plant haven at the same time, fruits and flowers in different stages of development; the different types of fruits are corresponding to different blooms.

The most important flowering, known commercially as "*Primofiore*", takes place from October until April and presents this characteristics: skin color from light green to yellow citrine; elliptical shape; medium size; fruit weight of not less than 100 gr; pale green or yellow citrine pulp; citrine yellow juice, with yields not less than 34%, acidity > 6%; pulp Brix > 7

To the massive blooming of April follows that between late May and early June.

Bianchetto o Maiolino (spring lemon) are the fruits collected from April to June and that meet the following specifications: yellow color skin; elliptical or ovoid shape; large size; fruit weight of not less than 100 g; yellow pulp; citrine yellow juice, with yields not less than 30%; acidity >5.5% ; pulp Brix > 6.5.

Verdello (summer lemon) are the fruits collected from July to September and that meet the following characteristics: light-green color skin; elliptical-globular shape; medium-large size; weight of not less than 100 g fruit; yellow citrine pulp.

At the end of winter (end of February-March) other flowers are formed from which arise the fruits called " *marzani* ".

Average lemon production in Italy was about 5.4 million quintals (18.2% of citrus fruit) and national production is concentrated mainly in Sicily (88.7%) and in Calabria and Campania.

Lemon growing in Sicily has become widespread in the provinces of Messina (28.3%), Catania (24.2%) and Palermo (23.7%), following to the importance province of Siracusa (18.3%).

The Italian heritage of lemon varieties consists:

Femminello

Its origin is unknown, but distant, in fact it is believed that the "Femminello" is one of the first introductions of lemons in Sicily. It is the cultivar that forms the basis of the Italian lemon, with several clones that behave differently in relation to productivity, the quality of the fruits and the resistance to desease.

The major clones are:

- ***"femminello comune"***

The "*Femminello commune"* is a clone of Femminello re-flowering with high capacity of fruit set. The fruit is very variable in size, shape, thickness of the peel, number seed, percentage and composition of the juice.

It is more or less rounded form, wrinkled skin, high acidity and more or less numerous seeds.

- ***"femminello sfusato"***

It is present mainly in the province of Siracusa. The characteristic that most distinguishes it from "*femminello comune*" is winter fruit, which is more elongated, with

prominent nipple, juicy, with high acidity and seeds rarely numerous. Also this cultivar provides several blooms and fructifications.

- ***"femminello santa teresa"***

It is more resistance to the dry mal than other clones of " *femminello* " but lower than that of " *monachello.* "

It was found in Santa Teresa Riva in province of Messina. The lack of dissemination is due to the rather shoddy on the fruit and low productivity.

- ***"femminello continella"***

The advantages of this product are high and constant production, seedless fruit, good yield juice and acidity, dry mal sensitivity lower sensitivity than other clones of "femminello."

- *"femminello zagara bianca"*

Probably originated from (for vegetative mutation) of the "Femminello comune", this prized cultivar, very tolerant to "mal secco", has a fruiting constant and high productivity can produce on average 18% of whitebait, 36% of verdelli and 46% of lemons, the latter two with good commercial skills.

Monachello

Fruit medium-small, elliptical but tapering at both ends; neck lacking; nipple small and inconspicuous; seeds few or none. Color yellow at maturity. Rind thin; surface smooth but with large sunken oil glands; very tightly adherent. Segments about 10; axis medium-small and solid. Flesh tender, somewhat lacking in juice, and acid content lower than most. Crop well distributed throughout year but mainly in winter and spring. Tree somewhat lacking in vigor, slow growing, and round-

topped; strongly drooping, slender, nearly thornless branches; dense foliage. Leaves large, thick, with undulate margins, and brighter green than most lemons. Fruit produced inside the foliage canopy. Moderately productive in comparison with Femminello and well adapted to forcing but with markedly reduced winter crop. The outstanding virtue of this distinctive Italian variety is its resistance to "mal secco" disease. This is the reason for its extensive planting some decades ago to the point where it was second only to Femminello. In all other respects, it is inferior to Femminello and currently it is planted only in areas where "mal secco" is very severe. Certain characteristics of this variety, particularly the distinctive growth habit and cross-sectional shape of the larger branches, suggest that it is a lemon-citron hybrid.

Interdonato

This cultivar is localized mainly in the province of Messina. Recently it has been spreading in Turkey and has been included in varietal assessment programs in other countries, mainly for the speed of enlargement of the fruit. The tree, vigorous and with sparse foliage, has a remarkable tolerance to dry mal and sore is sparsely re-flowering. The fruit is smooth with few seeds. The production is harvested in early autumn and parts over the last summer days.

QUALITATIVE ASPECTS

Lemon fruit [*C. limon* (L.) Burm. f.] contains many important natural chemical components, including phenolic compounds (mainly flavonoids) and other nutrients and non-

nutrients (vitamins, minerals, dietary fiber, essential oils and carotenoids). Their health-promoting effects and properties have been associated with their contents, namely vitamin C and flavonoids, due to their natural antioxidant characteristics (52,53).Overall, lemon fruits, rich in flavonoids, are a very important part of a balanced diet, particularly for their role in prevention of diseases, such as obesity, diabetes, blood lipid lowering, cardiovascular diseases, and certain types of cancer (54,55).

These compounds are not equally distributed in the lemon fruit. Hesperidin and eriocitrin occur mainly in lemon juice (56, 57, 58,59). In recent researches two isomers of hesperidin, neohesperidin and homoeriodictyol-7-O-rutinoside have also been identified in lemon juices.

The peel is richer in flavonoids than the seeds (60). Lemon seeds contain eriocitrin and hesperidin more abundantly and very low amounts of naringin. On the contrary, the peel is rich in neoeriocitrin, neohesperidin and naringin and has minor amounts of narirutin (61,62) In addition, the flavonoid concentrations in lemon fruits depend on the cultivar, maturity stage, etc. (56, 63)

Other phenolic compounds such as hydroxycinnamic acids are also known to be present in very low concentrations (caffeic, cholorogenic, ferulic, sinapic and p-coumaric acids) (64, 59, 65, 66), in addition to benzoic acids (protocatechuic, p-hydroxybenzoic and vanillic acids).

Lemon is a rich source of carotenoids, constituents that protect against photo-oxi damage (67). However, the concentration of carotenoids is strongly dependent on Citrus variety and

growing conditions (68). In recent years, many researches have focused on lemon in relation to the major carotenoid components of several orange species and orange juices showing that lemon contains reasonable quantities of carotenoids for a daily nutrition (69,70). Mature lemon accumulates β-cryptoxanthin (β-cry) as a principal carotenoid (71) and it accumulates predominantly in the flavedo and juice sacs in mature fruits (72).

Lemon is a rich source of vitamins for human diet, vitamin C being the main one present in this citrus. Other vitamins present in minor quantities are A and B-group (B1, B2, B3, B6 and B9) (73).

Vitamin C is highly bioavailable and is the most important water soluble antioxidant in cells as well as an efficient scavenger of reactive oxygen species with two biologically active forms: ascorbic acid (AA) and dehydroascorbic acid (DHAA) (74,75). The antioxidant function of vitamin C is based on its ability as hydrogen donor that lets it inactivate free radicals preventing proteins, lipid and DNA damages (76,77).

The main mineral present in lemon is potassium, although other minerals like calcium, magnesium and phosphorus are also present in minor levels. Moreover, lemon contains trace levels of copper, iron, manganese, selenium, sodium and zinc. Potassium constitutes an essential mineral for human health since it is essential to maintain the water–acid balance and it participates in the transmission of nerve impulse to muscle (78).

Lemon constitutes an interesting source of dietary fiber, also called non-starch polysaccharides (NSP), that may be classified as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF)

(79) The SDF/IDF ratio is fundamental for dietary and functional properties.

Lemon peel is the structure that presents the major content of dietary fiber [61] and pectin is the major component of fiber present in lemon. A reasonable dietary fiber intake is considered 25–30 g/day and lemon may constitute a valuable contribution to meeting the daily fiber requirements (78).

Lemons are consumed fresh and processed, as juices, jam, jellies, molasses, etc. The two main products of the citrus processing are juice and essential oil. Citrus factory extracts also from lemon bioactive compounds like flavonoids, vitamins, minerals, dietary fiber, etc. that are used in the food, cosmetic and pharmaceutical industry (80). Most of lemon by-products can be used as functional ingredients, in the development of healthy foods, the so-called "functional foods".

2.2 TRACEABILTY

The "food scandals" such as Bovine Spongiform Encephalopathy (BSE), dioxin crisis and the avian influenza have caused a major impact on consumption habits in the EU countries and a strong distrust condition towards the agri-food system.

Given these circumstances, to give a strong response, EU issued the *Regulation 178/2002* which provided the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food.

According to Regulation 178/2002 traceability is "*the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution.*"

The mentioned regulation is the fundamental law on food safety in Europe and, since its application (1st January 2005), the definition of a traceability system for the whole food sector, has become mandatory in all member countries. Reasons for the implementation of this system are many and can be resumed in two main points: protect public health and answer to consumers' demand of transparency, quality and safety.

This Regulation has been followed by several other regulations; for animal products the most important are 852/2004, 853/2004, 854/2004 and 882/2004, all of them corroborate the importance of a traceability system and the need to control them by authorities.

There are two categories of traceability that are commonly discussed under the same heading of traceability: internal traceability relating to the traceability of a product and the

information relating to it, within the company or factory. External traceability which relates to product information that a company either receives or provides to other members of the supply chain.

In recent years, food traceability has become a matter of great importance in relation to safety, quality and typicalness issues. In particular, the problem of authenticity assurance and adulterations' recognition arises beyond all others and its management calls for the possibility to identify products' provenance, through issues arising from the method of cultivation and by geographic origin for the purpose of protect both producers and consumers from frauds.

Organic agriculture is best known as a farming method where no synthetic fertilizers and pesticides are used.

According to the definition of the Codex Alimentarius, "*organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system.*"

According to the EC Regulation n. 834/2007, synthetic nitrogen fertilizers are not permitted in organic farming. Instead, soil fertility is maintained through the use of crop rotations that include green manures and also by the application of selected fertilizers which may be permitted where the need is recognized by an inspecting authority. Fertilizers that may be

permitted include animal manures, composts, and other products of plant and animal origin, e.g. rapemeal, bloodmeal, fishmeal, and seaweed products.

Generally, the analytical controls performed on organic fruits and vegetables consist of the search for pesticide residues. Thus, the absence or presence of these substances is used as a criterion to discriminate between organically and conventionally grown products. Anyway, to guarantee the consumer that a fruit is really organic, it is not enough to prove the absence of pesticides, because a product without pesticides does not always come from an organically managed farm. In organic production systems the nitrogen content of the organic fertilizers must be mineralized before being absorbed by the plants and the mineralization rate depends on many factors such as climate, soil type and microbiological activity. In addition, the chemical composition of the organic material applied will affect its decomposition and, hence, the nutrients available for crops. Thus, a risk for plants in organically managed farms is a low nitrogen availability that leads to negative effects on yield and fruit quality. To avoid this problem, unscrupulous farmers could apply synthetic nitrogen to the soil, for example, by fertirrigation. In this case, the fraud is difficult to detect (81).

It has been suggested that inputs of chemically synthesized nitrogen fertilizers, used in conventional agricultural regimes, may produce crops that can be differentiated on the basis of their nitrogen isotope composition from crops grown under organic regimes.

The possible use of nitrogen isotopes to differentiate between crops grown with or without inputs of synthetic nitrogen is based on the hypothesis that the application of synthetic nitrogen fertilizers, with $\delta^{15}N$ values close to 0‰, will result in the $\delta^{15}N$ of plants grown in conventional regimes being lower than those grown in organic regimes.

In 2005, Rapisarda et al. (81) carried out a study to verify the possibility to differentiate between organically and conventionally grown Navelina and Tarocco orange fruits. About the $^{15}N/^{14}N$ ($\delta^{15}N$) isotope ratio, fruits from organically managed farms had statistically higher $\delta^{15}N$ values in proteins of pulp and amino acids of the juice, compared to the conventional ones. Therefore, this have demonstrated that the application of organic fertilizers (uncomposted cattle manure or poultry manure and plant residues), which notoriously increases the level of ^{15}N in the soil (82), also determines an increase in $\delta^{15}N$ values in the organic citrus fruit (81).

As a result, this parameter have been considered new markers to differentiate organically from conventionally grown orange fruits.

Over the last decade consumers have shown a renewed interest in foods strongly linked to the place of origin, in particular the Food Standards Agency revealed that the labelling of foodstuffs with their country of origin is at the top of the list of requests for consumers. The reasons for this renewed interest has emerged from various reasons:

1) specific qualities and characteristics typical of culinary regional products;

2) health;

3) media attention;
4) distrust in the quality and safety of products coming out of their region, country or outside the EU;

The EU Regulation (2081/92) allows the application of the following geographical indications to a food – product: protected designation of origin (**PDO**, attributed *to those foods whose qualitative characteristics are essentially or exclusively from the territory where it is produced),* protected geographical indications (**PGI**, attributed *to those agricultural products and foodstuffs for which a given quality, reputation or other characteristic depends on the geographical origin and the production, processing and/or elaboration occurs in a geographical area*) and traditional speciality guaranteed (**TSG** *caters to agricultural products and foodstuffs that have a "special" linked to the method of production or to the composition related to the tradition of an area, but they are not necessarily products only in that area*).

Nowadays these denominations are regulated by Regulation EC 510/2006.

According the Regulation EC 510/2006 is defined as "origin designation" the name of a region, a determined place or, in exceptional cases, a village that is used to design an agricultural product:

• that takes origin in that region, determined place or village;
• whose qualities are essentially due to a particular geographic area,

including natural and human factors;

• whose production, processing and elaboration take place in that delimited geographic region;

With the adoption of these Regulations the EU intended to enhance and protect the quality and typicality of some productions; also, tried to meet the growing needs of consumer information and to support the rural economy, especially in marginal and disadvantaged areas of the European Union. The UE also wanted support the evolution of quality assurance systems based on the respect of specific production standards set by regulations that must be observed by manufacturers so that they can affix "PDO" or "PGI", identified and protected in the whole area of the UE. Standards respect to guarantee the impartiality and transparency of the procedures of supervision, is ensured by external certification bodies which, subject to the approval of the Member States, shall verify the conformity of products to the production regulations. This obviously represents a protection of typical products and at the same time of consumers.

New EU Regulation (N. 628/08) replace the previous and to meet a need, felt by many operators and consumers themselves, change the graphic symbols of PDO / PGI.

If in our country the EC Regulation of 2002 on food traceability is being widely applied (in accordance with these directions, each business operator must be able to show their customers and suppliers and have those systems and procedures to identify the product, so that it is easier for the withdrawal in the event of danger to the health of the consumer), it lacks a true commitment towards what has been called "traceability evolved", ie a wide range of methodologies that link to the monitoring of several production processes,

control of mixing techniques and processing of raw materials and the protection of the area of origin.

The development of innovative techniques and methods for the control of food products is a top priority in the development plans of both Community and national, to pursue the objectives of increasing security and protection of food quality. The use of non-destructive analytical methods, for the rapid application, for reporting fast, effective and high performance with respect to matrices investigated, if properly applied to verify the authenticity of product, represent a valuable and irreplaceable tool for the authorities responsible to perform control functions. In addition, scientific innovation and technological evolution of tools and methodologies, can help you quickly identify fraud and adulteration particularly sophisticated or specifically designed to evade inspection "of the law" currently applied.

Vast array of analytical techniques and parameters have been studied to verify the provenance of foods, such as aroma, sugar, phenolic and flavour compound profiling by gas and liquid chromatography.

Recently multi-element and isotopic analysis have been applied to a range of foodstuffs to develop methods that will permit their geographical origins to be determined with varying degrees of certainly.

Measuring elemental concentrations and isotopic variation in products is arguably the best analytical strategy for accurately verifying geographical origin. This approach is driven by the hypotheses outlined above describing the global variation of

isotope abundance of the “light” bio-element and “heavy” geo-element.

2.2.1 Sicilian typical citrus productions

Citrus fruits are typical Sicilian productions. In the last years the Sicilian citriculture is involved in a marked crisis due to a scarce technological expansion, along the whole chain production, and to the lack of a concrete commercial strategy for the valorization of productions. This crisis can be overcome only through the technological improvement of citriculture and the valorization of typical citrus productions.

Sicilian citrus fruits appreciated on foreign markets, thanks to their organoleptic and nutritional qualities, have received quality awards.

In the 2007 the Ribera orange has obtained the recognition PDO.

"*Riberella*" orange is a blonde pulp, seedless and pleasant flavour; unique qualities are essentially linked to environmental factors: climate, soil and water, in fact, the orange groves from which originate the oranges are present mainly on the sides and on the slopes of "Verdura" and "Magazzolo" rivers.

The PDO "**Arancia di ribera**" is limited to productions derived from variety: Brasiliano with clones: Brasiliano comune, Brasiliano risanato; Washington Navel, Washington Navel Comune, Washinton Navel risanato, Washington Navel 3033; Navelina with clones: Navelina comune, Navelina risanata e Navelina ISA 315.

In the 2009 "**Limone Interdonato**" has obtained the recognition PGI.

"*Interdonato*" is the trade name for a natural hybrid between a clone of cedar and a clone of lemon product in a vast area of the Ionian coast in the province of Messina. Represents the

traditional culture of the area and in recent years was revalued. The most important requirements of the product are the earliness of ripening (September – December) that allows placement in a period of high market demand and the particular characteristics of quality that make it particularly attractive for fresh consumption.

"**Mandarino tardivo di Ciaculli**" born spontaneously, for gemmary mutation from Mandarin Havana, owes its name to the locality where this mutation occurred, Ctrd. "Ricchizza" in Ciaculli. The strong aroma, high sugar content, the thin skin fine-grained, yellow orange color and the scarce presence of seeds (from 2 to 6), makes of this product an mandarin unique and unmistakable, particularly appreciated by consumers. It was characterized low calorie and high nutritional content.

For mandarins, recently was established the Consortium "Tardivo Ciaculli" but this product did not get yet the recognition IGP.

The typical citrus varieties described above occupy limited space in the contest of Sicilian citrus industry. However, the two most important species both for extensions of cultivation and commercial interest, are respectively:

PGI **"ARANCIA ROSSA DI SICILIA**" and PGI "**LIMONE DI SIRACUSA".**

2.2.2 PGI "Arancia Rossa di Sicilia"

"ARANCIA ROSSA DI SICILIA", represents one of the first Sicilian typical products protected at national level.

The production area of "ARANCIA ROSSA DI SICLIA" comprises the territory of eastern Sicily suitable for the cultivation of oranges and is thus identified:

-Province of Catania following Town district (Comuni) : Adrano, Belpasso, Catania, Biancavilla, Caltagirone, Castel di Judica, Grammichele, Licodia Eubea, Mazzarrone, Militello Val di Catania, Mineo, Misterbianco, Motta Sant'Anastasia, Palagonia, Paternò, Ramacca, Santa Maria di Licodia, Scordia and Randazzo.

-Province of Syracuse following Town district (Comuni): Francofonte, Lentini, Carlentini, Buccheri, Melilli, Augusta, Priolo, Siracusa, Solarino, Sortino Floridia.

-Province of Enna following communes: Santa Clara, Regalbuto, Catenanuova and Troina.

-Province of Ragusa following Town district (Comuni): Acate, Chiaramonte, Comiso and Vittoria.

Environmental conditions and culture of orange groves for the production of "ARANCIA ROSSA DI SICILIA" should

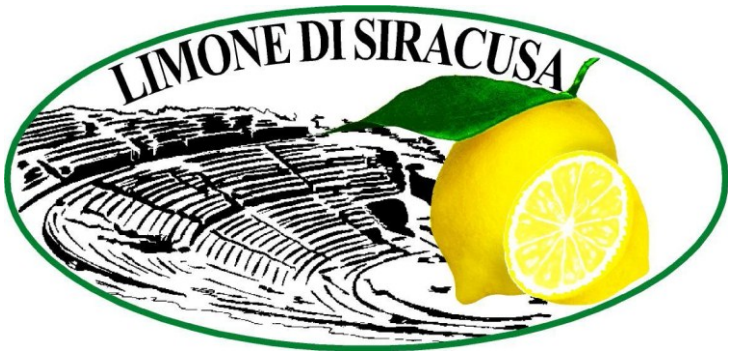
be traditional ones and, in any case, in order to give the resulting product specific quality characteristics the plant densities, types of farming and pruning systems are those in general use in order to maintain a perfect balance and development of the plant in addition to a normal aeration and insolation. The density of plants per hectare is normally between 230 and 420 for existing installations and intended for low density is permitted up to 725 plants per hectare. For sixth density dynamic is between 600 and 840 plants per hectare. The suitable rootstocks are: bitter orange, c*itrange Troyer*, *citrange Carrizo, Poncirus trifoliata*, free from virus diseases with high genetic stability.

The Sicilian blood orange is entered for consumption with the logo of PGI appearing on each fruit and packaged in accordance with the general and metrology rules of the fruit and vegetable trade. The packaging must bear, in clear, indelible characters and clearly distinguishable from all other writing and naming "ARANCIA ROSSA DI SICILIA", immediately followed by the varietal designation (Tarocco, Moro and Sanguinello). The space must appear immediately below the word "protected geographical indication". It is prohibited to add to the indication any qualification or other terms than those expressly provided for this production including adjectives: type, extra, above, selected, chosen, ecc. It is also prohibited to use names of varieties other than those expressly provided for this production.

It is however allowed to use designations that refer to company name or private labels, provided that they do not have laudatory meanings and are not likely to mislead the

purchaser such as name of orange groves from which actually originate the oranges, business name and address of the packer, gross weight.

2.2.3 PGI “Limone di Siracusa”



The PGI "Limone di Siracusa" is reserved to the cultivar "Femminello" and its clones grown in the province of Syracuse. The geographic area of cultivation of PGI "Limone di Siracusa" includes the Towns districts of Augusta and Melilli, Siracusa, Noto, Avola, Rosolini, Floridia, Solarino, Sortino and Priolo Gargallo. This area extends no more than 10 km from the Ionian Sea and does not exceed 210 metres above sea level and is bordered to the North and South respectively by south-facing valleys of the stream Porcaria and Tellaro river.

The cultivation method must be the one traditionally adopted in the area. The plant densities, types of farming and pruning systems must be designed to maintain a perfect balance and development of the plant in addition to a normal ventilation and daylight. The rootstocks are as follows: "*Citrus aurantium*” “*Poncirus trifoliata*", "*Citrange Troyer*”, “*Citrange Carrizo*" and "*Citrus macrophylla*”, equipped with high genetic stability. Cultivation operations, conventional technique management and harvest methods, shall be those laid down in "Normal Good Farming Practice". These regulations, for the

lemon, relate to the management of soil, fertilizers, irrigation and defense.

The production of PGI "Limone di Siracusa" can occur in systems with the method of cultivation:

a) *conventional* that is the one in use in the area, with the observance of the rules of "Normal Good Agricultural Practice" of the Sicilian region;

b) *integrated* with production achieved by compliance with the technical standards laid down in the product specification of the Sicilian region in adopting EU Regulations in agri-environment matters;

c) *organic*: in observance of Reg Ec 834/2007.

The harvest of the fruit from the plant must be carried out by hand using traditional methods at a level of development that ensures good organoleptic quality and aesthetics. It is admitted that the color of the fruit is variable depending on climatic and soil conditions-in accordance with various blooms and the harvest time. Maximum production of lemons is 29t/fixed for the whole campaign of production comprising the fruits of all the blooms.

It is compulsory to indicate legible and visible on at least one side of the packaging variety, source, category, size, lot. In retail sale signs laid down for the marking must be presented clearly and legibly. Products presented in prepackages in accordance with Directive 79/112/EC should be referred to the net weight, in addition to all the mentions required by legislation. Available packaging must be new and the materials must be cardboard, wood, plastic. Prohibited the use of plastic packaging, recyclable.

2.2.4 Multitechnique approach to geographical origin investigation

Generally, when investigating food provenance issues, it is essential to consider all the factors that may have influenced the composition of the food (age-variety- seasonality).

In the preceding 20-30 years a wide array of analytical techniques have been utilized in the development of methodologies for the characterization and authentication of foods. Parameters that have been used include bulk properties, such as pH, total N content, and trace element levels.

Recently has been seen how trace element profiling and heavy isotope ratio measurements reflect the underlying geohydrological environment on which the food was produced. Branche et al. (83) used a combination of two specifically selected elements (Cd and Se), heavy isotopic ratios (Pb and Sr), and light isotopic ratios (C and N) in an investigation into the geographical origin of wheat. The element were chosen for following reasons: Cd is reported to be a good indicator of anthropogenic activity and natural, geological occurrence, making it a useful determinant in geographical origin investigations, Se content of plant material grown on the North American continent is known to be elevated. The fractionation of elements C and N was influenced by environmental and varietal differences.

Day et al. (84) reported that, using a combination of certain trace elements and isotope ratios it was possible discern wine from large production areas around France.

Multitechnique approach has become also valuable tools in authenticity control and origin determination of fruit juices.

Quality of citrus fruit juice is defined by physical characteristics such as weight, yield of juice, color, odor and chemical parameters such as soluble solids content (SST), pH, acidity and content of bioactive compounds such as flavonoids, hydrossicinnamic acids and vitamins, which determine the taste and nutrition of citrus juices. These parameters are determine with different analytical techniques.

Recently, the development of spectroscopy techniques (NIR, IRMS, ICP-OES) and chemometrics (PCA, LDA) have resulted in rapid detection for chemical components and been widely applied in the field of food chemistry for identification of geographical origin of juice.

2.2.4.1 Near infrared spectroscopy

NIRS is a type of vibrational spectroscopy that employs photon energy (hv) in the energy range of 2.65 x 10-19 to 7.96 x 10-20 J, which corresponds to the wavelength range of 750 to 2500 nm (wave numbers: 13300 to 4000cm^{-1}) (85). (Fig.1)

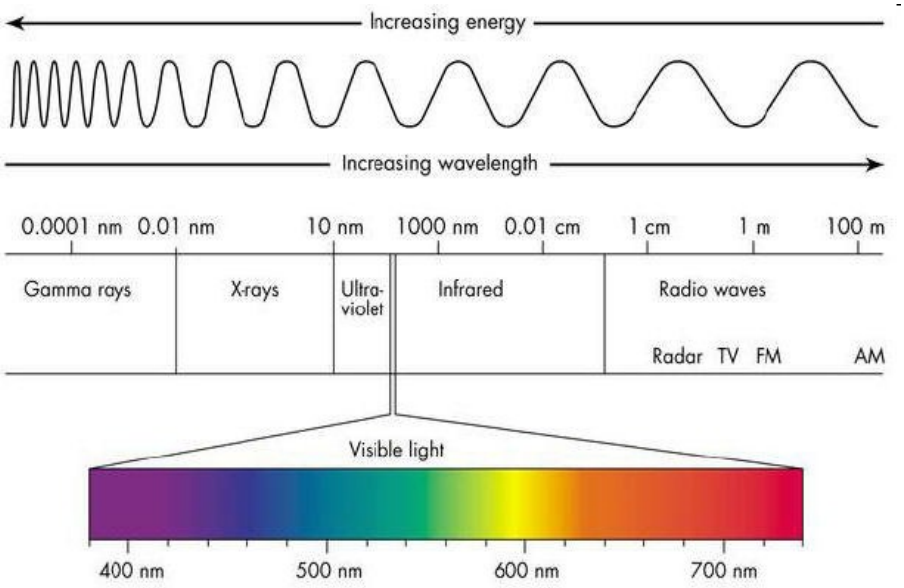


Figure 1: Electromagnetic spectrum

The basic principles of NIR spectroscopy involve the production, recording and interpretation of spectra arising from the interaction of electromagnetic radiation with the organic matter (86). The atoms of molecules, which give origin to organic matter, are linked together by several kind of bonds like the covalent ones. These bonds are subjected to vibration activities. At ambient temperature most of the molecules are in their fundamental vibration energy levels. The amplitudes of these vibrations are of a few nanometres and will increase if some energy is transferred to the molecule. This energy can be

transferred from a photon of a given wavelength (λ), for which the energy (Ep) can be given by:

$$E_p = h\nu = hc/\lambda$$

in which *h* is the Plank constant and *c* is the velocity of light. Radiation of a given frequency, capable to supply exactly the energy between two vibration levels or of their overtones or combination of two or more vibrations, can be absorbed by the molecule and can produce excitation to a higher vibration energy level. In the NIR wavelength range, some frequencies will be absorbed, other (that do not match any of the energy differences possible for that molecule) will not be absorbed while some will be partially absorbed. The absorption can only occur if the displacement of the atoms in a vibration mode can produce a change in the dipole moment of the molecule or in the local group of vibrating atoms. The intensity of a given absorption band is associated with the magnitude of the dipole change during the displacement of atoms in a vibration and with its degree of an harmonicity. Both phenomena are presented in great intensity associated with bonds involving the hydrogen atom and some other heavier element such as carbon, nitrogen and sulphur. Recording the response of certain molecular bonds (for example, O-H; N-H; C-H) to NIR radiation, generates a spectrum that may be characteristic of a sample and may act as a "fingerprint" (87) This spectrum is rich in chemical and physical information about organic molecules, and may therefore yield valuable information about the composition of a product (88).

NIR radiation interacting with a sample may be absorbed, transmitted or reflected. Thus, there are different modes of

measurements in NIR spectroscopy fitting different applications. In practise the common modes are transmittance, interactance, transflectance, diffuse transmittance, and diffuse reflectance, with the last two being the most frequently used (89).

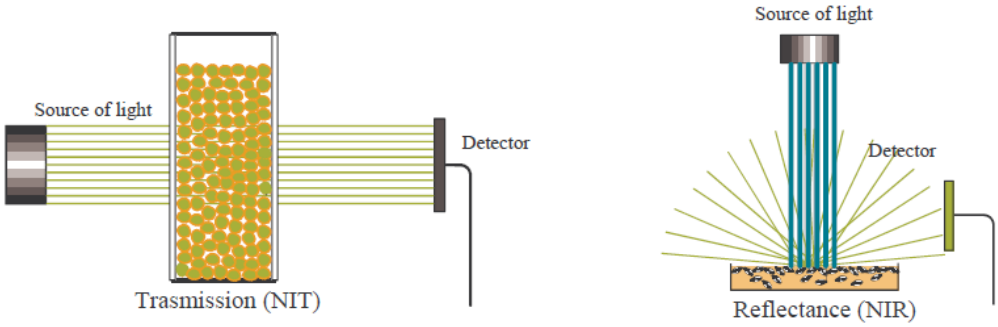


Figure 2: Most common scanning modalities in NIRS

The possible measurements modality to choose depends on the physical characteristics of sample like if solid or liquid, transparent or opaque and by the size of the particles.

The main difference between these scanning modalities is the position of the detector compared to the radiation beam and sample location. Transmission mode is considered the most frequently used and appropriate to assess internal quality attributes such as sugars and acids levels of thick-skinned fruit such as mandarin and orange (90, 91)

However, implementation of transmittance spectra measurement mode is limited by two fundamental factors. The first is the fact that transmittance spectra of fruit with different sizes, thickness of peel and shape have a different optical path and optical density. It is therefore expected that calibration model performance of spectra collected using transmission

optical geometry will be affected by size, shape and peel thickness.

Second, because of the low penetration depth of NIR radiation, the intensity of the incident NIR light needs to be very high to have at least some transmitted. Such high light intensity levels may cause permanent thermal damage to the fruit at the illuminated spot.

Reflectance spectra provide information about the appearance attributes, which are commonly accepted criteria for the product quality evaluation. In the reflectance mode, the field of view of the light detector includes parts of the fruit surface directly illuminated by the source, this being the easiest mode to obtain measurements, because they require no contact with the fruit and light levels are relatively high.

One of the advantages of reflectance mode is that measurements are easier to obtain and the light levels of the reflected radiation are much higher than that of transmitted radiation, however, variations in superficial and surface properties of the fruit may influence calibrations.

In interactance mode, the field of view of the detector is separated from the illuminated surface by a light seal in contact with the fruit surface. Basically, the relative merit of interactance mode is that it provides a compromise between reflection and transmission modes.

Transflectance is not popular as the transmission mode. However, it can be successfully used for a liquid stream, frequently in conjunction with optical bundle probes. It is suitable for in-line analysis and may be more appropriate for industrial application.

Near-Infrared spectroscopy has several advantages over other analytical techniques:

- the spectral measurements is really rapid (one sample can be scanned in less than 1 min);
- less expensive because there is not any use of chemical reagents and a single operator can analyze a large number of samples;
- several scans can be made on the same object, which permits to obtain a more representative sample composition and a more accurate result of analysis;
- sample require minimal (drying and grinding) or no preparation;
- several constituents of the same sample can be measured at the same time;
- easily applicable in different environments (like industry, laboratory, harvesters, etc.);
- measurements can also be carried out on/in/at line;
- the opportunity to use optical probes makes it possible to analyse the sample *in-situ,* the availability of portable instruments permits to obtain spectra directly in the field, useful to follow process like ripening.

On the other hand, NIR spectroscopy has some disadvantages to take into account:

- low sensitivity of the signal which can limit the determination of substances with concentration below 0.1%;
- it is a secondary analytical method, so it requires an accurate chemical an physical analysis as reference data;

- development of calibration models required high trained personnel;
- accurate and robust calibration requires a large data set incorporating large variation, which is often difficult to obtain;
- it requires a continuous maintenance of the calibration data set;
- although NIRS technique has low measuring costs, the initial high financial investment for the instrumentation represents an important obstacle for the purchase.

2.2.4.2 Isotopic Ratio Mass Spectrometry

Isotopic methodologies have become in recent years an increasing role both in interdisciplinary and applied research, and in an increasing number of analytical procedures and controls in the industrial, environmental, biomedical and food industries.

The use of fertilizers, of certain foods in the diet of farm animals, seasonal variations and geological factors (e.g., soil composition, altitude, etc.) influence the isotopic ratio and thus can be used to assign the source of regional agricultural products.

Food on which it is possible to apply this technique range from foods of animal origin as milk to those of vegetable origin, such as honey, wine, coffee and fruit juices.

The isotope ratios of all the elements in nature are, in the course of chemical and physical processes that characterize the evolution of the terrestrial ecosystem, appreciable effects of fractionation with modern techniques.

The carbon used by plants in natural photosynthetic processes, derives entirely from atmospheric CO_2, which, from the point of view isotope, is extremely homogeneous.

During the process of photosynthesis occurs isotope fractionation between atmospheric CO_2 and plant species, determining, in the latter, a depletion of the heavier isotope (^{13}C). This stock split is a function of the photosynthetic cycle followed by the plant, in almost all of the species due to the "Calvin cycle" (called "C3") and the "Cycle of Hatch and Slack" (called "C4"). (92) The content in ^{13}C characteristic of the two

cycles photosynthetic is very different, and therefore easily distinguishable from the point of view isotope.

The oxygen and hydrogen, as well as the carbon, represent the most abundant elements in the organic matter. Changes in the content of ^{18}O and ^{2}H in plant species are due to different and articulated processes and closely related to factors such as temperature, rainfall, water absorption from the soil by the roots, evapotranspiration, atmospheric oxygen, photosynthesis, etc.., factors that in turn are dependent on the local climate (93).

2.2.4.3 Induced Coupled Plasma - OES

The primary source of nutrients required for the healthy and successful propagation of a plant (and the animal that feed on it) is the soil and water on which it has grown.

This in turn, is a reflection of the underlying geology of the area within the organisms immediate vicinity

Therefore, it is not surprising that the mineral composition of a food is widely used as a means of identifying its geographical origin, as for example, in the case of wine (94,95,96), rice (97,98), dairy products (99), wheat (100) and orange juice (101).

However it is not just the presence or absence of an element that is useful (as most element will be present at some concentration) but it is the relative variation in the trace element profile that is the parameter that provides the major discrimination power.

The introduction of inductively-coupled plasma-optical emission spectrometry (ICP-OES) allowed a wider range of elements to be analysed.

McHard et al. (102) were possibly some of the first researchers to apply a normalization procedure to multielement data in order to maximize the differences between two sets of samples. Their approach, which is now accepted as being a standard tool for use in chemometric investigations, was to identify an element whose concentration was constant, irrespective of the geographical origin of the samples, and then to normalize all other elemental data against it.

In McHard's study on fruit juice, they used Zn.

The simple calculation, used by McHard to ratio elemental data from the fruit juice, is as follows:

$$(I_{AY}/I_{AR})/(I_{BY}/I_{BR})$$

where A is the element under consideration, B is the reference element, *I* is the intensity reading for the respective element, Y is the sample being analyzed and R is the reference sample

This approach has three main advantages:

- it acts as an internal standardization procedure, negating the need for calculations to be performed to compensate for other sample attributes, such as solids content, matrix interferences, specific gravity.
- it simplifies data, allowing elements present at low concentrations to be easily visualized alongside element present at many orders to magnitude higher.

2.2.4.4 Chemometric Analysis

The discipline that enables you to extract information relevant data from chemically produced in a series of numerous experiments called "Chemometrics" and relies on the use of mathematical methods - statisticians. Generally, the real systems that are under observation and from which you want to draw an information, are of multivariate type, i.e. they are governed simultaneously by multiple variables. Only rarely are univariate type. Until the advent of modern computers most procedures and analytical statistics did not take account of this fact and tended to turn in univariate problems, even those that are inherently multivariate. Chemometrics, instead, allows a multivariate approach to the process to be studied: in this way makes it possible to account for all the variables in play, allowing you to make the best use of any information contained in the data to be analyzed.

Prerequisite for a good statistical data analysis is that these are numerous, good quality or are relevant to the issue that you want to analyze, have little noise and have as much information as possible. As regards the latter condition, it is important that the number of samples from which are then extrapolated the data is adequate and that the samples themselves are representative of the population that goes to analyze.

Data are generally arranged in arrays (arrays brute) where the rows correspond to the various samples (or cases) and columns correspond to variables; then each sample consists of c variables. The values of c variables identify the location of the sample in c-space dimensions.

2.2.4.4.1 ***Principal component analysis***

Principal components analysis (PCA) aims to reduce the original data array to an array smaller than that still keeps the same information. Often, in fact, are the changes a variable than the other (co-variance) to contain the most relevant information. A complete representation of the problem would require a display of samples into a space with a rank equal to the number of variables used. It is obvious that if you have more than three variables, as often happens, a graphical representation would be impossible. The PCA solves this problem by using a compression of information that allows a useful and immediate visualization of the data. Principal components analysis aims to identify, from the data available, the privileged directions along which concentrates the highest variance. In this way you will get new abstract variables, such as main components.

These new directions, linear combinations of the original ones, are the principal components (PC) or Eigenvectors.

The PCs reduce the data in a way that maximises between sample spectral variation. A set of n spectra can be expressed as a n * p data matrix X containing n values of transmission absorbance at each of the p wavelength. The general equation for PC calculation (103) is

$$X= TP^{T} + E$$

Where T is the score matrix,

P is the transposed eigenvector matrix,

E is the residual matrix.

The scores are a new values of spectra in the coordinate system defined by PCs and the eigenvector are the link between the wavelengths of the X matrix, and the principal component space (103)

Each eigenvalue, in summary, expresses the variance explained by single principal component and can be expressed as a percentage. Generally the last main components are just informative and contain most of the "noise".

2.2.4.4.2 ***Linear discriminant analysis***

Linear discriminant analysis (LDA) is a supervised classification technique where the number of categories and the samples that belong to each category are previously defined (103, 104).

Linear discriminant analysis is the simplest classification method and also the most widely used. Classification methods are intended to build a model that can identify the classification of each sample on the basis of a number of independent variables. Unlike the cluster analysis and principal components analysis, classification methods require that classes are known a priori, and that for a number of samples known to belong to class so he can build the model.

The method produces a number of orthogonal linear discriminant functions equal to the number of categories minus one, that allow the samples to be classified in one or another category (104)

References

1. Peri C. Qualità nelle aziende e nelle filiere agroalimentari. Hoepli, Milano, **2004**.

2. Kapsak W.R., Schmidt D., Childs N.M., Meunier J., White C. "Consumer perceptions of graded, graphic and text label presentations for qualified health claims."*Critical Reviews in Food Science and Nutrition,* **2008**, 48(3):248-256.

3. Liu S., Manson J.E., Lee L.M., Cole S.R., Hennekens C.H., Willett W.C., Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. *American Journal of Clinical Nutrition,* **2000,** 72 (4), 922-928

4. Martin A., Cherubini A., Andres-Lacueva C., Paniagua M., Joseph J.A. Effects of fruits andvegetables on levels of vitamin E and C in the brain and their association with cognitive performance. *The Journal of Nutrition Health and Aging,* **2002**, 6 (6), 392-404

5. Sauco, V.G. Mango production and word market: current situation and future prospects. *ISHS Acta Horticulturae*, **2004**, 645, VII International Mango Symposium, 1, 107-116

6. Mossler, M.A., & Nesheim, N.O. Florida crop/pest management profile: Mango. University of Florida: Cooperative Extension Services in the Institute of Food and Agricultural Sciences, **2002**

7. Ali, KH.M. Factors influencing the quality and shelf-life of Mangodeen. MSc. Thesis. Dep. of Fd. Sci. and Technol. U. of K. Khartoum, Sudan, **2003**

8. Nagy, S. and Shaw, P. Tropical and sub-tropical fruits (composition properties and uses) *Avi Publishing Inc. West port.* Florida, **1980**, 187-220

9. Hulme, A.C. Biochemistry of fruits and their products. Vol. II. Mangoes dehydrated fruits. Academic Press. London and New York, **1971**, 233-234, 623-648

10. Saleh N.A.M., El-Ansari M.A.I. Polyphenolics of twenty local varieties of *Mangifera Indica*. *Planta Medica,* **1975**, 28, 124-130

11. Gao, L., & Mazza, G. Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *Journal of Agricultural and Food Chemistry,* **1995**, 43, 343-346.

12. Liu, Y., Liu, X., Zhong, F., Tian, R., Zhang, K., Zhang, X., & Li, T. Comparative study of phenolic compounds and antioxidant activity in different species of cherries. *Journal of Food Science,* **2011**, 76, 633-638.

13. Usenik, V., Fajt, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., & Veberic, R. Sweet cherry pomological and biochemical characteristics influenced by rootstock. *Journal of Agricultural and Food Chemistry,* **2010**, 58, 4928-4933.

14. Gonçalves, B., Silva, A. P., Moutinho-Pereira, J., Bacelar, E., Rosa, E., & Meyer, A. S. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). *Food Chemistry,* **2007**, 103, 976-984.

15. Kelebek, H., & Selli, S. Evaluation of chemical constituents and antioxidant activity of sweet cherry (*Prunus avium* L.) cultivars. *International Journal of Food Science & Technology,* **2011**, 46, 2530-2537.

16. Mazza, G., & Brouillard, R. The mechanism of co-pigmentation of anthocyanins in aqueous solutions. *Phytochemistry,* **1990**, 29, 1097 1102.

17. Mozetic , B., Trebse, P., & Hribar, J. Determination and quantitation of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Goricaregion (Slovenia). *Food Technology and Biotechnology,* **2002**, 40, 207–212.

18. Liu, Y., Liu, X., Zhong, F., Tian, R., Zhang, K., Zhang, X., & Li, T. Comparative study of phenolic compounds and antioxidant activity in different species of cherries. *Journal of Food Science,* **2011**, 76, 633-638.

19. Usenik, V., Fabcic, J., & Stampar, F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chemistry,* **2008**, 107, 185-192.

20. Serra, A. T., Duarte, R. O., Bronze, M. R., & Duarte, C. M. M. Identification of bioactive response in traditional cherries from Portugal. *Food Chemistry,* **2011**, 125, 318-325.

21. Kang, S-Y., Seeram, N. P., Nair, M. G., & Bourquin, L. D. Tart cherry anthocyanins inhibit tumor development in Apc^{Min} mice and reduce proliferation of human colon cancer cells. *Cancer Letters,* **2003**, 194, 13–19.

22. Jacob, R. A., Spinozzi, G. M., Simon, V. A., Kelley, D. S., Prior, R. L., Hess-Pierce, B., & Kader, A. A. Consumption of cherries lowers plasma urate in healthy women. *Journal of Nutrition,* **2003,** 133, 1826–1829.

23. Seeram, N. P., Momin, R. A., Nair, M. G., & Bourquin, L. D. Cyclooxygenase inhibitory and antioxidant cyanidin

glycosides in cherries and berries. *Phytomedicine,* **2001**, 8, 362–369.

24. Kim, D. O., Heo, H. J., Kim, Y. J., Yang, H. S., & Lee, C. Y. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *Journal of Agricultural and Food Chemistry,* **2005**, 53, 9921–9927.

25. Kinsella J.E., Frankel E.N., German J.B., Kanner J. Possible mechanism for the protective role of antioxidants in wine and plants foods. *Food Technol.,* **1993**, 47, 85-89

26. Cao G., Russell R.M., Lischner N., Prior R.L. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamina C in elderly women. *J.Nutr.,* **1998,** 128, 2383-2390

27. Jang M., Cai L., Udeani G.O., Slowing K.V., Thomas C.F., Beecher C.W., Fong H.S., Farnsworth N.R., Kinghorn A. D., Mehta R.G., Moon R.C., Pezzuto J.M. Cancer chemopreventive acitivity of resveratrol, a natural product derived from grapes. *Science,* **1997,** 275, 218-220

28. Renaud S.C., Gueguen R., Schenker J., D'Houtaud A. Alcohol and mortality in middle-aged men form Eastern France. *Epiodemiology*, **1998,** 9, 184-188

29. Dorozynski A. Wine may prevent dementia *Br. Med. J.*, **1997,** 314, 997

30. Del Nobile MA, Baiano A, Benedetto A, Massignan L Respiration rate of minimally processed lettuce as affected by packaging. J *Food Eng.*, **2006**, 74:60–9

31. Lee L, Arul J, Lencki R, Castaigne F. A review on modified atmosphere packaging and preservation of fresh fruits

and vegetables: physiological basis and practical aspects-part 2. *Packaging Technol Sci*., **1996,** 9:1–17

32. Kou, L.P., X.H. Liu, Y.G. Luo. Effects of mild heat treatment and serving of rachis on quality, decay rate and shelf-life of fresh-cut grapes. Proceedings of the sixth international symposium on viticulture and enology. Peoples R China, **2009**, 20-22, 103-113.

33. Ortiz, J. M. Botany: taxonomy, morphology and physiology of fruits, leaves and flowers. In *Citrus: The Genus Citrus*. Dugo, G.; Di Giacomo, A. CRC Press, **2002**; 16-31

34. Widmer, W. W.; Montanari, A. M. The potential for citrus phytochemicals in hypernutrition foods. In *Hypernutritious Foods*; Agscience Inc. Auburndale, Fla., **1996**; 75-89.

35. Gadjeva V., Kuchukova D., Georgieva R. Vitamin combinations reduce oxidative stress and improve antioxidant status in patients with iron deficiency anemia. *Comp. Clin. Path*., **2005**, 14, 99-104.

36. Vannozzi G. Manuale di Scienza dell'Alimentazione. La nuova Italia Scientifica, Roma **1993,** 67.

37. Cook J.D. and Monsen E.R. Vitamin C, the common cold and iron absorption. American *Journal of Clinical Nutrition*, **1977,** 38, 648–659

38. Hazell T.and Johnson. In vitro estimation of iron availability from a range of plant foods: influence of phytate, ascorbate and citrate. British Journal of Nutrition, **1987**, 57, 223–233.

39. Hurrell R.F. Prospects for improving the iron fortification of foods. In S. Faman, & S. Zlotkin *Nutritional anemias*, **1992**, 193–208. New York: Raven Press.

40. Horowitz, R. M. The Citrus flavonoids. In *The Orange. Its Biochemistry and Physiology*. Sinclair W. B., Ed. University of California, Division of Agricultural Sciences, **1961**; 334-372.

41. Wilmsen, P. K.; Spada, D. S.; Salvador, M. Antioxidant activity of flavonoid hesperidin in chemical and biological systems. *J. Agric. Food Chem*., **2005**, 53, 4757-4761.

42. Attaway, J. A. Citrus juice flavonoids with anticarcinogenic and antitumor properties. *In Food Phytochemicals for Cancer Prevention I*, Maple Press: York, PA, **1994**; 240-248.

43. Galati, E. M.; Monforte, M.T.; Kirjavainen, S.; Forestieri, A. M.; Trovato, A. Biological effects of hesperidin, a Citrus flavonoid. (note I): antiinflamatory and analgesic activity. *Il Farmaco,* **1994**, 49, 709-712.

44. Natella, F.;Nardini, M.; Di Felice, M.; Scaccini, C. Benzoic and cinnamic acid derivatives as antioxidants structure – activity relation. *J. Agric. Food Chem*., **1999**, 47,1453-1459.

45. Andreasen, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa M.T.. Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *J. Agric. Food Chem*., **2001**. 49, 5679-5684.

46. Hrazdina G. Anthocyanins. The Flavonoids. Advances in Research, Harborne, Mabry, Ed., Chapman and Hall, London and New York, **1982**, 169.

47. Maccarone, E.; Rapisarda, P.; Fanella, F.; Arena, E.;Mondello, L. Cyanidin-3-(6″-malonyl)-β-glucoside. One of

the major anthocyanins in blood orange juice. *Ital. J. Food Sci*., **1998**, 4, 10, 367-372.

48. Rapisarda, P.; Russo, G. Fruit quality of five Tarocco selections grown in Italy. *Proc. Int. Soc. of Citriculture,* **2000**, 1149-1153.

49. Mazza, G.; Miniati, E. Anthocyanins in fruit, vegetables and, grains; CRC Press Inc.: Boca Raton; FL, **1993.**

50. Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem*., **1997**, 45, 304-309.

51. Kähkönen, M. P.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycons. *J. Agric. Food Chem*., **2003**, 51, 628-633

52. Proteggente A.R., Pannala A.S., Paganga G., Van Buren L., Wagner E., Wiseman S., Van De Put F., Dacombe C., Rice-Evans C.A., *Free Radical Res*., **2002**, 36, 217–233.

53. Wilmsen P.K., Spada D.S., Salvador M., *J. Agric. Food Chem.,* **2005**. 53, 4757–4761.

54. Benavente-García O., Castillo J., *J. Agric. Food Chem*., **2008**, 56, 6185–6205.

55. Vanamala J., Reddivari L., Yoo K.S., Pike L.M., Patil B.S., *J. Food Compos. Anal*., **2006**, 19, 157–166.

56. González-Molina E., Moreno D.A., García-Viguera C., J. *Agric. Food Chem*., **2008**, 56, 1669–1675.

57. Miyake Y., Suzuki E., Ohya S., Fukumoto S., Hiramitsu M., Sakaida K., Osawa T., Furuichi Y., *J. Food Sci*., **2006**, 71, S633–S637.

58. Caristi C., Bellocco E., Panzera V., Toscano G., Vadala R., Leuzzi U., *J. Agric. Food Chem*., **2003**, 51, 3528–3534.

59. Wang Y.C., Chuang Y.C., Ku Y.H., *Food Chem*., **2007**, 102, 1163–1171.

60. Jaime L., Molla E., A. Fernandez, M.A., Martín- Cabrejas J., Lopez-Andreu, Esteban R.M., *J. Agric. Food Chem*., **2002**, 50, 122–128.

61. Baldi A., Rosen R.T., Fukuda E.K., Ho C.T., *J. Chromatogr. A,* **1995**, 718, 89–97.

62. Kawaii S., Tomono Y., Katase E., Ogawa K., Yano M., *J. Agric. Food Chem*., **1999**, 47, 3565–3571.

63. Vandercook C.E., Tisserat B., *Phytochemistry,* **1989**, 28, 799–803.

64. .Wang Y.C, Chuang Y.C., Hsu H.W., *Food Chem*., **2008**, 106, 277–284.

65. Bocco A., Cuvelier M.E., Richard H., Berset C., *J. Agric. Food Chem.,* **1998**, 46, 2123–2129.

66. Manthey J.A., Grohmann K., *J. Agric. Food Chem*., **2001**, 49, 268–3273.

67. Goodwin T.W., *Food Chem*., **1980**, 5, 3–13.

68. Gross J., *Food Sci. Technol.,* **1987,** 167–191.

69. Gil-Izquierdo A.,. Gil M.I, Ferreres F., *J. Agric. Food Chem.,* **2002**, 50, 5107–5114.

70. Melendez-Martínez A.J., Britton G., Vicario I.M., Heredia F.J., *Food Res. Int.,* **2005**, 38, 931–936.

71. Kato M., Ikoma Y., Matsumoto H.,. Sugiura M, Hyodo H., Yano M., *Plant Physiol*., **2004**, 134, 824–837.

72. Ikoma Y., Komatsu A., Kita M., Ogawa K., Omura M.,. Yano M, Moriguchi T., *Physiologia Plantarum*, **2001** 111, 232–238.

73. Penniston K.L., Nakada S.Y., Holmes R.P., Assimos D.G, *J. Endourol.,* **2008**, 22, 567–570.

74. Halliwell B., *Free Radical Res.*, **1996** 25, 439–454.

75. Halliwell B., *Free Radical Res*., **1996** 25, 57–74.

76. Gown A.M., Tsukada T., Ross R., *Am. J. Pathol*., **1986** 125, 191–207.

77. Harats D., Chevion S., Nahir M., Norman Y., Sagee O., Berry E.M., *Am. J. Clin. Nutr*., **1998**, 67, 240–245.

78. E.N.a.S.R.R. Whitney, Understanding Nutrition, Eighth ed., **1999**.

79. Gorinstein S., Martín-Belloso O., Park Y.S., Haruenkit R., Lojek A., Iz M., Caspi A., Libman I., Trakhtenberg S., *Food Chem.*, **2001,** 74, 309–315.

80. Ozaki Y., Miyake M., Inaba N., Ayano S., Ifuku Y., Hasegawa S., Limonoid glucosides of Satsuma mandarin (Citrus unshiu Marcov.) and its processing products, *ACS Symp. Ser*., **2000**, 107–119.

81. Rapisarda, P., Calabretta M. L., Romano, G., Intrigliolo, F. Nitrogen Metabolism components as a tool to discriminate between organic and conventional citrus fruits, *J. Agric. Food Chem*., **2005,** 53, 2664-2669,

82. Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardiux, A., Tardiux, P. Experimental determination of nitrogen kinetic isotope fractionation: some principles, illustration for the denitrification and nitrification processes. *Plant Soil*, **1981,** 62, 413-430.

83. Branch S., Burke S., Evans P., Fairman B., Wolff Briche C.S.J. A preliminary study in determining the geographical origino f wheat using isotope ratio inductively coupled plasma mass spectrometry with ^{13}C, ^{15}N mass spectrometry. *J. Anal. Atom. Spectrom*., **2003**, 18, 17-22

84. Day M.P., Zhang B.L., Martin G.J. Determination of the geographical origin of wine using joint analysis of elemental and isotopic composition. II – Differentiation of the principal production zones in France for the 1990 vintage. *J. Sci. Food Agric.,* **1995,** 67, 113-123

85. Pasquini, Celio. Near Infrared Spectroscopy for Laboratory Process Monitoring. *The NIR SPECTRUM,* **2003**, *1(4):4-7.*

86. Manley, M., Downey, G., Baeten, V., Spectroscopic technique: Near-Infrared (NIR) spectroscopy. In: Modern techniques for food authentication. Da-Wen Sun 1st ed., Amsterdam; Boston: *Elsevier/Academic Press*., **2008**, 65-115.

87. Woodcock T et al., Confirmation of Declared Provenance of European Extra Virgin Olive Oil Samples by NIR spectroscopy . *Journal of Agricultural and Food Chemistry,* **2008**, 56 (23), 11520-11525.

88. Katsumoto Y., Jiang J., Berry R. J., Ozaki Y. Modern pretreatment methods in NIR spectroscopy. *Near Infrared Anal.,* **2001,** 2: 29-36.

89. Huang, W.N., M.C. Olewnik, J.J. Psotka and R.E. Dempster. Measuring rheological dough and mixing properties of full formula ingredients and micro-ingredients in real time using NIR. **2001** AACC Annual Meeting. Charlotte

90. Kawano S., Fujiwara T., Iwamoto M. Non-destructive determination of sugar content in "Satsuma" mandarins using NIRS transmittance. *Journal of the Japanese Society for Horticultural Science,* **1993**, 62, 465-470

91. Miyamoto K., Kitano Y. Non- destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmission spectroscopy *J. NIR Spectrosc*., **1995** 3, 227-237

92. Krueger H., W., Reesman R. H. Carbon isotope analyses in food technology. *Mass Spectrometry Reviews,* **1982**, 1,205-236

93. Kelly S., Heaton K., Hoogewerff J. Tracing the geographic origin of food: the application of multi-element and multi-isotope analysis. *Trends in Food Science*, **2005,** 16, 407-409

94. Kwan W-O., Kowalski B.R., Skogerboe R.K. Pattern recognition analysis of element data. Wines of *vitis vinifera* cv. Pinot noir from France and the United States, *J. Agric. Food Chem*., **1979,** 27, 1321-1326

95. Baxter M.J., Crews H.M., Dennis M.J., Goodall I., Anderson D. The determination of the authenticity of wine from its trace element composition *Food Chem.,* **1997**, 60, 443-450

96. Perez-Trujillo J.P., Barbaste M., Medina B. Contents of trace and ultratrace elements in wines from Canary Islands (Spain) as determined by ICP-MS. *J. Wine Res*., **2002**, 13, 243-256

97. Yasui A., Shindoh K. Determination of the geographic origin of brown-rice with trace-element composition. *Bunseki Kagaku,* **2000**, 49, 405-410

98. Kelly S., Baxter M., Chapman S., Rhodes C., Dennis J., Brereton P. The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. *Eur. Food Res. Technol*., **2002**, 214, 72-78

99. Rossmann A., Haberhauer G., Holzl S., Horn P., Pichelmayer F., Voerkelius S. The potential of multielement stable isotope analysis for regional origin assignment of butter. *Eur. Food Res. Technol*., **2000,** 21, 32-40

100. Branch S., Burke S., Evans P., Fairman B., Wolff Briche C.S.J. A preliminary study in determining the geographical origin of wheat using isotope ratio inductively coupled plasma mass spectrometry with ^{13}C, ^{15}N mass spectrometry. *J. Anal. Atom Spectrom*., **2003,** 18, 17-22

101. Day M.P., Correia P., Hammond D.A. ^{13}C-IRIS: an improved method to detect the addiction of low levels of C_4-derived sugars to juices. *J. Assoc. Off. Anal. Chem*., **2001**, 84, 957-963

102. McHard J.A., Foulk J.F., Winefordner J.D. A comparison of the trace element content of Florida and Brazil orange juice. *J. Agric. Food Chem*., **1979**, 27, 1326-1328

103. Otto, M. Chemometrics, **1999,** 314 Wiley-VCH. Weinheim, Germany.

104. Naes, T., T. Isaksson, T. Fearn, and T. Davies. A user-friendly guide to multivariate calibration and classification. NIR Publications, **2002**, 344 Chichester, UK.

3. PUBLICATIONS AND WORKS IN PROGRESS

3.1 QUALITY OF MEDITERRANEAN FRUITS: NUTRITIONAL AND TECHNOLOGICAL ASPECTS

- **MANGO**

PUBL.1

Guarrasi V., Barone F., San Biagio P.L., **Amenta M**., Rapisarda P., Germanà M.A. "Studio preliminare sulle caratteristiche qualitative e salutistiche di 4 cultivar di mango (MANGIFERA INDICA L.) coltivate in Sicilia". Dal libro Qualità e tipicità degli alimenti mediterranei a cura di G. Dugo, G. Di Bella, N. Cicero, V. Lo Turco, R. Rando, pp.367-371.

- **SWEET CHERRY**

PUBL.2

Gabriele Ballistreri, Alberto Continella, Alessandra Gentile, **Margherita Amenta,** Simona Fabroni, Paolo Rapisarda. "Fruit quality and human health- bioactive compounds of sweet cherry (Prunus avius L.) cultivars grown in Italy". Food Chemistry 2012.11.024 doi 10.1016

PUBL.3

Amenta M., Fabroni S., Cutuli M., Gugliuzza G., Rapisarda P., Quality and functional properties of cherry fruits cv. "Mastrantonio" in progress.

PUBL.4

Gabriele Ballistreri, Alberto Continella, Alessandra Gentile, **Margherita Amenta**, Simona Fabroni, Paolo Rapisarda. "Fruit quality and human health- bioactive compounds of sweet cherry (*Prunus avius L.*) cultivars grown in Italy" presentato a *Chimalsi - IX Congresso Italiano di Chimica degli Alimenti,* 03-07 Giugno 2012 Ischia.

PUBL.5

Amenta M., Fabroni S., Cutuli M., Gugliuzza G., Rapisarda P., "Qualità e proprietà funzionali di frutti di ciliegio cv Mastrantonio" presentato al VIII Congresso Nazionale di Chimica degli Alimenti "Qualità e tipicità degli Alimenti Mediterranei : Alimentazione e Salute", 20-24 Settembre 2010 Marsala (PA).

- **TABLE GRAPE**

PUBL.6

E. Nicolosi, F. Ferlito, **M. Amenta**, P. Rapisarda "Shelf- life of minimally processed table grapes pace in snack-size containers". Accepted for publication to Acta Horticolturae

- **CITRUS FRUIT**

PUBL. 7

Supercritical carbon dioxide – treated blood orange juice as a new product in the fresh fruit juice market. Simona Fabroni,

Margherita Amenta, Nicolina Timpanaro, Paolo Rapisarda. *Innovative Food Science and Emerging Technologies* 11, 477-484

PUBL. 8

P. Rapisarda, **M. Amenta**, S. Fabroni, C. Licciardello,D. Pietro Paolo, S. Recupero, G. Rizza, V. Rizzo,M.P. Russo, G. Russo, G. Reforgiato Recupero. "Variabilità dei componenti salutistici nelle arance bionde e pigmentate". *Rivista di Frutticoltura e di ortofloricoltura Anno LXXIV N.1-2 pag.64-70*

PUBL.9

Fabroni Simona, **Amenta Margherita**, Sanfilippo Laura, Rapisarda. Nuovi agrumi con elevate proprietà funzionali. Paolo. Dal libro Qualità e tipicità degli alimenti mediterranei a cura di G. Dugo, G. Di Bella, N. Cicero, V. Lo Turco, R. Rando, pp.308-314.

PUBL.10

Fabroni S., **Amenta M**., Rizza G. and Rapisarda P. Anthocyanins in citrus, presentato all' "XII International Citrus Congress" tenutosi dal 18-23 Novembre 2012 a Valencia (Spain).

PUBL.11

Fabroni S., **Amenta M**., Todaro A., and Rapisarda P. Change in non volatile flavours of blood and common fruits during cold storage, presentato all' "XII International Citrus Congress" tenutosi dal 18-23 Novembre 2012 a Valencia (Spain).

PUBL.12

Simona Fabroni, **Margherita Amenta**, Giorgio Rizza, Valeria Rizzo, Giuseppe Russo, Paolo Rapisarda. "New triploid citrus hybrids: quality and functional properties of fruits" presentato a Chimalsi - IX Congresso Italiano di Chimica degli Alimenti, 03-07 Giugno 2012 Ischia.

PUBL.13

Paolo Rapisarda, Simona Fabroni, **Margherita Amenta,** Giorgio Rizza, Nicolina Timpanaro "Changes in sensory quality of blood and common orange fruits during cold storage", presentato a ALIMED 2011 Mediterranean Diet Congress: Quality, Safety and Health, 22-25 maggio 2011, Palermo (PA).

PUBL.14

Paolo Rapisarda, **Margherita Amenta**, Simona Fabroni, Valeria Rizzo, Nicolina Timpanaro, Maria Allegra e Antonio Leonardi "Qualità e aspetti salutistici di frutti di clementine biologici e convenzionali" presentato al IX Giornate Scientifiche SOI, 10-12 Marzo 2010 Firenze.

3.2 TRACEABILITY

PUBL.15

Margherita Amenta, Simona Fabroni, Maria Allegra, Guido Sorrentino, Giancarlo Roccuzzo, Paolo Rapisarda. "Authentication of the Geographical Origin of Sicilian Typical Citrus Fruits through a Chemical and Chemometric Approach", in press

PUBL.16

Fabroni S., **Amenta M**. and Rapisarda P. Authentication of the geographical origin of Sicilian typical *citrus* productions, presentato all' "XII International Citrus Congress" tenutosi dal 18-23 Novembre 2012 a Valencia (Spain).

PUBL.17

Margherita Amenta, Simona Fabroni, Giorgio Rizza, Paolo Rapisarda "Authentication of the geographical origin of Sicilian typical citrus productions" presentato a *Chimalsi - IX Congresso Italiano di Chimica degli Alimenti,* 03-07 Giugno 2012 Ischia.

PUBL.1


Dipartimento di Scienze degli Alimenti e dell'Ambiente
"Prof. G. Stagno d'Alcontres"
VIII
Congresso Nazionale di Chimica degli Alimenti
Qualità e Tipicità degli Alimenti Mediterranei: Alimentazione e Salute
20 - 24 Settembre 2010
Marsala
Società Chimica Italiana
Riassunti
a cura di
Giuseppa Di Bella, Vincenzo Lo Turco,
Nicola Cicero e Angela Giorgia Potortì

STUDIO PRELIMINARE SULLE CARATTERISTICHE QUALITATIVE E SALUTISTICHE DI 4 CULTIVAR DI MANGO (*MANGIFERA INDICA* L.) COLTIVATE IN SICILIA

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INTRODUZIONE

Il mango (*Mangifera indica* L.) originario dell'India, è ampiamente coltivato in numerose zone calde del mondo ed in Sicilia vi sono alcune particolari località caratterizzate da condizioni pedoclimatiche favorevoli per il suo sviluppo [1]. In particolare nelle province di Catania, Palermo e Messina si coltivano differenti cultivar di mango che coprono un arco temporale di maturazione che va da agosto con la cultivar più precoce ('Glenn') fino a novembre con la cultivar più tardiva ('Keitt'). I frutti del mango sono utilizzati freschi o trasformati e risultano particolarmente apprezzati per la succosità e la dolcezza della polpa. In bibliografia sono presenti alcuni lavori che impiegano i Sistemi Olfattivi Elettronici per determinare in maniera non distruttiva la maturità di frutti di mango [2], ma pochi sono ancora i lavori in cui questi vengono impiegati per discriminare il pattern aromatico tra le cultivar.

Nel presente lavoro sono stati determinati i parametri classici della qualità, i componenti dotati di proprietà nutrizionali e salutistiche, l'attività antiossidante e la componente volatile di 4 cultivar di mango, coltivate in Sicilia. Inoltre, è stata effettuata la discriminazione qualitativa dell'aroma delle cultivar in oggetto mediante un Sistema Olfattivo Elettronico ('naso elettronico').

MATERIALI E METODI

La prova è stata condotta nel 2009 su frutti provenienti da un campo commerciale ubicato in provincia di Messina. Sono state prese in esame le 4 cultivar di mango: 'Irwin', 'Glenn', 'Kensington Pride' e 'Maya' e per ogni cultivar sono stati raccolti 4 frutti su cui sono stati rilevati i parametri carpometrici, quali il peso ed i diametri equatoriale e longitudinale. Inoltre, sono stati determinati secondo metodi standard parametri chimico-fisici quali l'acidità totale (AT) e i solidi solubili totali (SST) [3]. La determinazione del contenuto di acido ascorbico è stata effettuata mediante metodo HPLC [4] , utilizzando un cromatografo liquido Waters mod.600E interfacciato ad un rivelatore a serie di fotodiodi (PDA) Waters 996 e gestito dal software Millenium 32 Waters. La colonna impiegata è stata una C18 Hypersil 250mm x 4.6mm x 5µm (Phenomenex, Torrence, CA), mantenuta alla temperatura di 35°C e l'eluizione è stata eseguita con acido ortofosforico 0.02 M con un flusso di 1.0 ml/min. La lunghezza d'onda è stata fissata a 260 nm. Dieci gr di purea sono stati trattati con una soluzione 3% di acido metafosforico (100 ml). Il campione è stato centrifugato a 5000 rpm per 20 min e filtrato usando un filtro da 0,45 µm prima dell'analisi HPLC. I carotenoidi totali sono stati determinati spettrofotometricamente a λ=450 nm, dopo estrazione dei pigmenti da 10g di purea con 20 ml di

soluzione esano:acetone:etanolo (50:25:25) ed espressi come mg di β-carotene/100g di prodotto fresco [5]. I polifenoli totali del succo sono stati valutati attraverso il saggio di Folin-Ciocalteau (FC) [6]. Un campione di succo (0,5 ml), ottenuto dopo centrifugazione della purea, è stato diluito in 10 ml di acqua e successivamente 1 ml di questa soluzione è stato miscelato con 5 ml di reagente FC (precedentemente diluito con acqua 1:10 v/v) e 4 ml di soluzione di carbonato sodico 7,5%. È stata effettuata la lettura spettrofotometrica a λ=740 nm ed il contenuto in polifenoli totali è stato espresso come mg di acido gallico/l di succo. L'attività antiossidante è stata misurata mediante il saggio ORAC [7], con lievi modifiche. Le misure sono state effettuate con uno spettrofluorimetro Wallac1420 Victor III 96, dotato di una piastra di lettura (EG &Wallac, Turku, Finland) con un filtro di fluorescenza (eccitazione 485 nm, emissione 535 nm). La reazione è stata condotta a 37°C a pH 7.0, usando come standard il Trolox (10μM) e 75mM di tampone fosfato come bianco. I valori di ORAC sono stati espressi come μmoli di Trolox equivalenti/100g di polpa.

L'elaborazione statistica dei risultati è stata effettuata mediante l'analisi della varianza (ANOVA), utilizzando il software STATSOFT 6.0.

Per caratterizzare la componente volatile delle 4 cultivar in oggetto sono stati determinati i profili molecolari con un sistema GCMS-QP2010S (Shimadzu, Tokyo, Japan), equipaggiato con colonna capillare SLB^{TM}–5ms da 30m x 0.25mm x 0.25μm (Supelco, Bellefonte, PA). Per il campionamento è stata adottata la tecnica dell'adsorbimento su fibra dello spazio di testa (HS-SPME), utilizzando fibre in Polidimetilsiloxano da 100 μm x 1cm (Supelco, Bellefonte, PA). Il metodo analitico utilizzato è stato messo a punto precedentemente da Guarrasi et al. (2010) [8]

L'indagine sull'aroma dei frutti è stata effettuata mediante Naso Elettronico. Nel presente studio è stato impiegato l'analizzatore sensoriale EOS^{835} (Sacmi, Imola, Italia), dotato di 6 sensori MOS aspecifici [7], capaci di estendere la loro sensibilità ad un largo spettro di composti volatili. Lo spazio di testa dei campioni da analizzare è stato preparato come sopra. Dopo il condizionamento termico, 4 ml di spazio di testa sono stati prelevati e successivamente introdotti nella camera di misura dell'EOS^{835} con l'ausilio di un autocampionatore (HT200H, HTA s.r.l., Italia). Per l'interpretazione dei risultati, le curve di risposta dei sensori sono state trasformate in variabili univoche (*feature*), elaborate mediante Analisi delle Componenti Principali (PCA), ovvero analisi statistica multivariata, non supervisionata, basata su Matrice di Correlazione. La *feature* calcolata per ogni sensore ed utilizzata nell'analisi statistica realizzata mediante il software del sistema (*Nose Pattern Editor*) è la differenza tra la resistenza elettrica del sensore in assenza delle sostanze volatili e la resistenza dello stesso sensore misurata in presenza delle sostanze volatili (R_0-R).

RISULTATI

I risultati delle analisi effettuate sui frutti di mango, sia per quanto concerne i parametri qualitativi che nutrizionali e salutistici sono riportati nella tab. 1. Il livello di acidità totale significativamente più basso è stato rilevato nella cv. 'Glenn', con differenze significative nei confronti della cv. 'Kensington Pride'. I valori di pH hanno seguito un andamento opposto. I solidi solubili totali sono risultati più elevati nella cv. 'Maya'. Questa cultivar ha mostrato anche livelli di acido ascorbico e carotenoidi molto superiori rispetto alle altre cultivar. Per quanto riguarda i componenti fenolici, che determinano l'astringenza dei frutti, la loro concentrazione risulta statisticamente più elevata nella cv. 'Maya' con valori di 367.74mg/l

eq. di acido gallico. Questo parametro, congiuntamente all'alto tenore di acido ascorbico e carotenoidi, ha influenzato i valori dell'attività antiossidante, che nella cultivar suddetta è risultata di 1871µmolTE/100g, con differenze significative rispetto alle cv. 'Irwin' e 'Kensington Pride'. Infine, è da mettere in evidenza, l'elevato valore di attività antiossidante rilevato nella cv. 'Glenn', non correlato al livello dei componenti antiossidanti determinati in questo studio, che sono risultati equivalenti o addirittura più bassi delle altre cv. Uno studio più approfondito su altri componenti ad azione antiossidante, come ad esempio il livello di vitamina E o di alcuni composti fenolici che hanno maggiore reattività verso il radicale utilizzato per la misura delle unità ORAC, potrebbe fornire maggiori informazioni sui valori registrati.

Nella caratterizzazione dell'aroma mediante gas cromatografia, sono state rivelate ed identificate 50, 34, 41 e 44 sostanze volatili (dati non riportati), rispettivamente per le cv. 'Glenn', 'Irwin', 'Kensington Pride' e 'Maya'. Tra queste è importante notare la determinante presenza percentuale del δ-3-carene, in special modo nelle cv. 'Glenn', 'Irwin' e 'Maya', molecola spesso abbondante nell'aroma del mango [9], anche se con una dimostrata scarsa attività olfattiva [10]. Nella cv. 'Kensington Pride', invece, una percentuale più alta è stata riscontrata di α-terpinolene, così come riportato per i frutti di questa cv. coltivati in Australia [11].Tale molecola è ben nota nell'aroma del mango e presenta, al contrario, una comprovata attività olfattiva con specifico sentore di 'dolce' [12]. Altri monoterpeni e sequiterpeni sono stati identificati, come ad esempio d-limonene, α-pinene, β-mircene, α-ocimene, β-phellandrene, β-cayophyllene, α-bergamotene, α-gurjunene, germacrene D, β-selinene, molti dei quali ritenuti responsabili di sentori olfattivi quali 'erbaceo', 'floreale', 'speziato', 'di bosco' [13]. Per quanto riguarda gli esteri sono stati identificati l'etil acetato e l'etil butanoato, considerati tra i principali responsabili dell'aroma dei frutti di mango [14]; il primo tra le molecole dell'aroma di 'Glenn', 'Kensington Pride' e 'Maya', il secondo, invece, esclusivamente nella cv. 'Irwin' con una percentuale significativamente più alta rispetto agli altri esteri. In Fig.1 sono rappresentate le sommatorie delle aree dei picchi delle singole molecole (come percentuali relative) suddivise per classe chimica.

L'array di sensori MOS impiegato per la valutazione dell'aroma è risultato sensibile al pattern aromatico del mango. L'analisi statistica multivariata (PCA) applicata alle risposte dei sensori ha presentato discriminazione con una percentuale di varianza totale nelle prime due componenti principali di circa l'87.9%, con predominanza di discriminazione sulla prima componente principale (PC1) (Fig. 2). Probabilmente pur essendo aspecifici, i sensori hanno percepito una differenza nell'aroma delle cv. in riferimento non alle singole molecole, ma alla classe chimica di appartenenza delle stesse. Sulla PC1, infatti, è pensabile una proporzionalità diretta nei confroni delle percentuali complessive dei terpeni (65.0% per 'Maya' e 'Irwinn', 72.5% per 'Glenn', 78.6% per 'Kensington Pride'). Al contrario invece la PC1 sembrerebbe presentare una proporzionalità indiretta per quanto concerne le percentuali degli Esteri (31.5% per 'Maya', 27.2% per 'Irwinn', 21.1% per 'Glenn', 20.9% per 'Kensington Pride').

CONCLUSIONI

Dal presente studio è emerso che i frutti di mango prodotti in Sicilia possono essere considerati un'ottima risorsa di composti bioattivi quali polifenoli, carotenoidi e vitamina C, tutte sostanze dotate di spiccate proprietà antiossidanti e salutistiche. In particolare, la cv. 'Maya' è risultata la varietà più ricca

di sostanze nutraceutiche. Le cultivar in oggetto, inoltre, hanno presentato un variegato pattern aromatico. Molte delle molecole individuate sono olfattivamente attive e rievocano sentori di erba fresca, bosco, frutta matura e fiori. Pertanto, la coltivazione del mango in Sicilia potrebbe diventare una valida alternativa alle tradizionali produzioni, poichè questo fruttifero sub-tropicale ha dimostrato di adattarsi bene alle condizioni pedoclimatiche di alcune zone dell'isola. Naturalmente il mercato premia i prodotti che raggiungono elevati standard qualitativi, ed il presente studio pone indubbiamente le basi per future e più approfondite valutazioni in merito alla produzione di qualità di mango siciliano.

RINGRAZIAMENTI

Si ringrazia sentitamente Pietro Cuccio della Cupitur per aver fornito i frutti oggetto della presente ricerca.

BIBLIOGRAFIA

[1] A. DE MICHELE, F. CALABRESE, F. BARONE, Rivista di frutticoltura e di ortofloricoltura 67 (6), 64–65 (2005).

[2] M. LEBRUN, A. PLOTTO, K. GOODNER, M.N. DUCAMP, E. BALDWIN. Postharvest Biology and Technology, 48, 122–131 (2008).

[3] AOAC – Association of Official Analytical Chemistry, *Official methods of analysis* (1990).

[4] P. RAPISARDA, S. INTELISANO. Italian Journal of Food Science, 8, 251–256 (1996).

[5] E.D. RITTER, A.E. PURCELL. In J.C. Bauernfeind, *Carotenoids as colorant and Vitamin A precursors*, 815–923 (1981). New York: Academic Press.

[6] V.L. SINGLETON, J.A. ROSSI, American J. Enol. Viticulture, 16, 144–158 (1965).

[7] B. OU, M. HAMPSCH-WOODILL, R. PRIOR, Journal of Agricultural and Food Chemistry, 49, 4619–4626 (2001).

[8] V. GUARRASI, V. FARINA , P.L. SAN BIAGIO , M.A. GERMANÀ. Italus Hortus, 17 (3), 103-108 (2010).

[9] J.A. PINO, J. MESA, Y. MUNOZ, M.P. MARTI, R. MARBOT, Journal of Agricultural and Food Chemistry, 53, 2213–2223 (2005).

[10] G. CAO, H. ALESSIO, R. CULTER, Free Radic Biol Med, 14 (3), 303–311 (1993).

[11] J.P. BARTLEY, Biomed.Environ.Mass Spectrom., 16, 201–205 (1988).

[12] J. PINO, J. MESA, Flavour Fragrance Journal, 21, 207–213 (2006).

[13] A.J. MACLEOD, C.H. SNYDER, Journal of Agricultural and Food Chemistry, 33,380–384 (1985).

[14] D.C. LOPES, S.R. FRAGA, C.M. REZENDE, Quim. Nova., 22, 31–36, (1999).

Tab.1

	Cultivar			
	GLENN	IRWIN	KENSINGTON PRIDE	MAYA
PESO (g)	416.2 ± 82.4 ns	432.3 ± 72.1 ns	520.2 ± 36.1 ns	606.1 ± 70.5 ns
DIAMETRO LONG. (mm)	11.1 ± 0.8 ns	10.8 ± 0.8 ns	11.3 ± 0.6 ns	12.2 ± 0.5 ns
DIAMETRO EQUAT. (mm)	8.1 ± 0.5 ns	8.3 ± 0.6 ns	8.8 ± 0.3 ns	9.0 ± 0.3 ns
pH	6.1 ± 0.1 A	5.4 ± 0.1 B	5.2 ± 0.1 B	5.7 ± 0.1AB
ACIDITÀ TOTALE (g/l)	0.26 ± 0.01 B	0.30 ± 0.01 AB	0.32 ± 0.01 A	0.30 ± 0.01 AB
SOLIDI SOLUBILI TOTALI (%)	15.61 ± 0.06 CB	16.35 ± 0.02 B	15.30 ± 0.01 C	18.02 ± 0.26 A
POLIFENOLI TOTALI (mg/L GAE)	294.88 B	216.90 C	304.76 B	367.74 A
ACIDO ASCORBICO (mg/100 g)	23.66 B	20.14 CB	17.67 C	41.05 A
CAROTENOIDI (mg/100 g)	3.07C	4.09 B	2.95 C	6.11 A
UNITÀ ORAC (µmol TE/100)	1864 A	1639 B	1152 C	1871 A

I valori per ogni singolo parametro seguiti da lettere diverse sono significativamente differenti tra di loro per p≤ 0.01 (ANOVA).

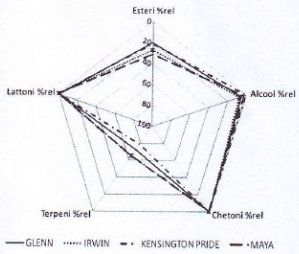

Fig.1 Analisi qualitativa dei picchi gascromatografici suddivisa per classi chimiche.

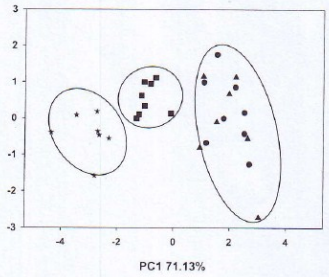

Fig.2 Discriminazione della componente aromatica delle 4 cultivar di *Mangifera indica* L., ottenuta mediante PCA delle risposte dei sensori MOS del l'analizzatore sensoriale EOS^{835}.

PUBL.2

ARTICLE IN PRESS

Food Chemistry xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Sweet cherry (*Prunus avium* L.)
Physicochemical characteristics
Sugars
Organic acids
Phenolic compounds
Anthocyanins
Antioxidant activity
ORAC

ABSTRACT

The fruit quality characteristics, phenolic compounds and antioxidant capacities of 24 sweet cherry (*Prunus avium* L.) cultivars grown on the mountainsides of the Etna volcano (Sicily, Italy) were evaluated. High-performance liquid chromatographic methods were used to identify and quantify sugars, organic acids and phenolics. A total of seven phenolic compounds were characterised as hydroxycinnamic acid derivatives (neochlorogenic acid, *p*-coumaroylquinic acid and chlorogenic acid) and anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-rutinoside and peonidin 3-rutinoside). The total anthocyanin content ranged from 6.21 to 94.20 mg cyanidin 3-glucoside equivalents/100 g fresh weight (FW), while the total phenol content ranged from 84.96 to 162.21 mg gallic acid equivalents/100 g FW. The oxygen radical absorbance capacity (ORAC) assay indicated that fruit of all genotypes possessed considerable antioxidant activity. The high level of phenolic compounds and antioxidant capacity of some sweet cherry fruits implied that they might be sources of bioactive compounds that are relevant to human health.

1. Introduction

Sweet cherry (*Prunus avium* L.) is one of the most popular temperate fruits. According to the FAO Statistical Database (FAO, 2010), the world production of sweet cherries is 2,102,651 t. The top worldwide producer is Turkey (417,905 t), followed by the United States of America (287,305 t), and Iran (255,500 t). Producing about 6% of the cherries in the world (115,476 t), Italy is the fourth largest producer (FAO, 2010). Sweet cherries have high commercial importance in Sicily, where there has been strong development over the last 30 yrs due to the introduction of new varieties and new orchards. On the mountainsides of the Etna volcano, three different typical varieties of sweet cherry, such as Donnantonio or Mastrantonio, Napoleona and Maiolina, are cultivated. The fruits of these cultivars are well known and appreciated by consumers for their characteristic sweetness, skin colour and firmness.

Sweet cherries are an excellent source of many nutrients and phytochemicals in addition to contributing to a healthy diet. They contain various phenolic compounds including hydroxycinnamate, flavonols, procyanidins and anthocyanins (Gao & Mazza, 1995; Liu et al., 2011; Usenik et al., 2010). The major anthocyanins identified in cherry fruits were the 3-*O*-glucoside and 3-*O*-rutinoside of cyanidin, with peonidin-3-*O*-rutinoside as well as pelargonidin-3-*O*-rutinoside being present in much lower amounts (Gonçalves et al., 2007; Kelebek & Selli, 2011). Sweet cherries are also rich in phenolic acids such as hydroxycinnamic acid derivatives (neochlorogenic acid, *p*-coumaroylquinic acid and chlorogenic acid) (Liu et al., 2011; Usenik et al., 2010). These components are important for their potential contribution to the colour of the cherry fruits through co-pigmentation with anthocyanins (Mazza & Brouillard, 1990; Mozetic, Trebse, & Hribar, 2002). Moreover, strong correlations were found between the phenolic content and antioxidant activity of fruits (Serra, Duarte, Bronze, & Duarte, 2011; Usenik, Fabcic, & Stampar, 2008).

It has been demonstrated that the consumption of sweet or sour cherries reduces the risk of cancer (Kang, Seeram, Nair, & Bourquin, 2003) as well as pain from arthritis and inflammation (Jacob et al., 2003; Seeram, Momin, Nair, & Bourquin, 2001) and offers protection against neurodegenerative diseases (Kim, Heo, Kim, Yang, & Lee, 2005).

The chemical composition of sweet cherries largely affects the sensory quality of fruits (Fazzari et al., 2008). Sweetness and skin colour influence consumer acceptance of cherry cultivars, as do fruit weight and firmness.

Many studies have reported on the physical, chemical and nutritional properties of sweet cherries (Esti, Cinquanta, Sinesio,

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0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.11.024

Please cite this article in press as: Ballistreri, G., et al. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chemistry* (2012), http://dx.doi.org/10.1016/j.foodchem.2012.11.024

Moneta, & Di Matteo, 2002; Faniadis, Drogoudi, & Vasilakakis, 2010; Girard & Kopp, 1998; Liu et al., 2011; Mozetic et al., 2002; Serra et al., 2011; Serrano et al., 2009; Usenik et al., 2010). However, there has been no detailed research on the chemical composition of sweet cherries grown in Italy. The present investigation evaluated fruit quality parameters (fruit weight, firmness, pH, soluble solids content, titratable acidity, colour, sugars and organic acids), hydroxycinnamic acid derivatives and total and individual anthocyanins as well as antioxidant activities by ORAC assay of 24 sweet cherry (*P. avium* L.) cultivars grown on the mountainsides of the Etna volcano (Sicily, Italy). We carried out this study with the goal of finding the most promising sweet cherry genotypes with respect to fruit quality and health-promoting components in order to develop a selection procedure suitable for a sweet cherry breeding program in Sicily.

2. Materials and methods

2.1. Materials

The fruits of sweet cherry cultivars were collected in 2011 from the experimental orchards of San Giovanni Montebello and Contrada Ragagli located in Giarre and Mascali, respectively (Sicily, Italy). The study included the fruits of 24 cultivars: Black Star, Blaze Star, Burlat, Donnantonio, Ducignola Nera, Early Star, Ferrovia, Gabbaladri, Genovese, Giorgia, Grace Star, Maiolina Grappolo, Maredda, Minnulara, Moreau, Napoleona Forestiera, Napoleona Grappolo, Napoleona Verifica, Puntalazzese, Sunburst, Sweet Early, Sweet Heart, Toscana and Zio Peppino. The cherries were picked at commercial maturity. For each cultivar, 3 replicates (consisting of 10 fruits each) were carried out ($n = 3$) and used for physicochemical analyses.

2.2. Chemicals

Hydroxycinnamic acids (*p*-coumaric acid and chlorogenic acid), organic acids (malic acid, shikimic acid, and fumaric acid), Folin–Ciocalteu's phenol reagent, AAPH (2,2′-azobis(2-methylpropionamidine)dihydrochloride), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), and fluorescein, were purchased from Sigma–Aldrich (Milan, Italy). Cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin chloride and pelargonidin chloride were purchased from Extrasynthèse (Genay, France). All other chemicals were of analytical grade, and the solvents used for chromatography were HPLC grade (Merck KGaA, Darmstadt, Germany).

2.3. Physicochemical analyses

Fruit weight was evaluated in triplicate from a 10 whole fruit sample (fruit and seed). The cherry seeds were manually removed with care and then the juices of the pitted cherries were extracted with a domestic blender (Bravosimac FS 300, Simac, Italy). Titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1 using an automatic pH titration system (Mettler DL 25, Mettler Toledo, Switzerland) and expressed as % of malic acid. The pH was measured with the same equipment used for TA, while the soluble solids content (SSC) was determined with a refractometer (Atago Rx 5000, Atago Co. Ltd., Japan) and expressed as °Brix. On the basis of the measured data, the SSC/TA ratio was calculated. Texture analysis was performed by measuring the maximum shear force using a Texture Analyzer (TR 53205, TR Turoni S.r.l., Italy) fitted with a stainless steel probe of 1/4 cm diameter and a length of 5 cm. Firmness was reported as the force in newtons (N) needed to penetrate 5 mm of cherry fruit. Four measurements were performed on two opposite sides of each sample (fruit). Colour values were measured from the surface (skin) of the cherry fruit with a portable colorimeter (CM-2500d, Minolta Co. Ltd., Japan) using D65 as the illuminant. The CIE (Commission International de l'Eclairage) colour parameters L^* (lightness), a^* (redness), b^* (yellowness), C^* (chroma) and h (hue angle) were measured. Four readings were collected for each sample.

Individual sugars (glucose, fructose and sorbitol) and organic acids (malic acid, shikimic acid and fumaric acid) were determined by HPLC. A sample of pitted fruits was homogenised with a domestic blender (Bravosimac FS 300, Simac, Italy) and an aliquot (10 g) was dissolved in 50 ml of twice distilled water and extracted for 10 min in an ultrasonic bath. Then, the solution was centrifuged (15,000 rpm for 20 min at 4 °C) and passed through a Sep-Pak C_{18} cartridge (Waters Corporation, Milford, MA) and a 0.45 μm filter (Albet, Barcelona, Spain). Separation and identification of sugars was carried out using a Waters 600-E HPLC system (Waters Corporation, Milford, MA) equipped with a Waters 410 refractive index (RI) detector. A Luna-NH_2 column (250 × 4.6 mm i.d., 5 μm; Phenomenex, Torrance, CA) was used and the elution was carried out with an acetonitrile:water (80:20 v/v) solution at a flow rate of 1.8 ml/min. HPLC analysis of organic acids was performed using a Waters 600-E equipped with a Waters 996 photodiode array detector (PDA). The column was a Synergi Polar-RP (150 × 4.6 mm i.d., 4 μm; Phenomenex, Torrance, CA). The solvent consisted of 4 mmol aqueous sulphuric acid, and separation was performed with a flow rate of 0.7 ml/min and detection at 210 nm. The sugars and organic acids were identified by comparing their retention times with the pure standards and confirmed by co-injection. Quantification of each compound was performed using an external standard calibration curve.

2.4. Extraction and determination of phenolic compounds

Phenolic compounds were extracted according to the method of González-Gómez et al. (2010) with some modification. Five grams of fresh cherry tissue were ground to homogenate and extracted three times using 25 ml of methanol solution (containing 0.5% of hydrochloric acid). The homogenate sample was centrifuged at 12,000 rpm for 15 min at 4 °C and filtered with a 0.45 μm filter (Albet, Barcelona, Spain) before HPLC analysis.

2.5. HPLC analysis of phenolic compounds and anthocyanins

The HPLC system was a Surveyor HPLC (Thermo Electron, San Jose, CA) equipped with a Surveyor photodiode array plus detector (PDA), controlled by ChromQuest 4.2.34 software. The individual phenolics and anthocyanins in the cherry extracts were detected at 320 and 520 nm, respectively. The column used was a Luna C_{18} (250 × 4.6 mm i.d., 5 μm; Phenomenex, Torrance, CA), maintained at 35 °C, for both the analysis of phenolics and anthocyanins. For the phenolic compounds (hydroxycinnamic acid derivatives), the eluents used were water:acetic acid (98:2 v/v) (A) and methanol (B) with a gradient transition from 95% to 70% of solvent (A) over 35 min at a flow rate of 1 ml/min. The phenolic compounds were identified by comparing their retention times and UV spectra with those of certified standards. The quantities of neochlorogenic acid were assessed from peak areas and calculated as equivalents of chlorogenic acid. Also, *p*-coumaroylquinic acid is a *p*-coumaric acid derivative, so the concentration of *p*-coumaroylquinic acid was calculated as equivalents of *p*-coumaric acid (Usenik et al., 2008). For the anthocyanins analysis, the elution solvents were water:formic acid:acetonitrile (87:10:3 v/v/v) (A) and water:formic acid:acetonitrile (40:10:50 v/v/v) (B). The percentage of (B) increased linearly from 6% to 90% in 45 min at a flow rate of 1 ml/min. The anthocyanins were identified by comparing their retention times and UV spectra with those of certified standards.

Please cite this article in press as: Ballistreri, G., et al. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chemistry* (2012), http://dx.doi.org/10.1016/j.foodchem.2012.11.024

The relative percentages of the individual anthocyanins were based on peak areas. The concentrations of the identified anthocyanins, expressed as mg of cyanidin 3-glucoside equivalents (CGE)/100 g FW, were calculated by comparing their percentages to the total anthocyanin content obtained by the pH differential assay (see below).

2.6. Determination of total anthocyanin and total phenol content

The total anthocyanin content in the cherries was assayed by the pH differential method (Rapisarda, Fanella, & Maccarone, 2000). The absorbance values of appropriately diluted cherry extracts were measured at 520 nm by a UV–vis spectrophotometer (Varian Cary 100 Scan, Palo Alto, CA). The total anthocyanin content was expressed as mg of cyanidin 3-glucoside equivalents (CGE)/100 g FW.

The total phenol content in cherries was determined according to the Folin–Ciocalteu (FC) colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Five grams of fresh cherry tissue were ground to homogenate and extracted by 25 ml of methanol, using an ultrasonic bath. Appropriately diluted cherry extracts (1 ml) were mixed with 5 ml of FC commercial reagent (previously diluted with water, 1:10 v/v) and 4 ml of a 7.5% sodium carbonate solution. The mixture was stirred for 2 h at room temperature away from strong light. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm and the total phenol concentration was expressed as mg of gallic acid equivalents (GAE)/100 g FW.

2.7. Evaluation of antioxidant activity

An oxygen radical absorbance capacity (ORAC) assay was used to evaluate the antioxidant capacity of the cherry samples (Ou, Hampsch-Woodill, & Prior, 2001). The measurements were carried out on a Victor III 96-well plate reader (PerkinElmer, Waltham, MA) with a fluorescence filter (excitation wavelength: 485 nm; emission wavelength: 535 nm); the Wallac 1420 software was used to control the system. Fluorescein (116 nmol) was the target molecule for free radical attack from AAPH (153 mmol), which was used as the peroxyl radical generator. The antioxidant activities were expressed as μmol of Trolox equivalents (TE)/100 g FW.

2.8. Statistical analysis

The results were compared by the one-way analysis of variance (ANOVA) using the Statgraphic Plus 4.1 software (Manugistics Inc., Rockville, MD). Duncan's multiple-range tests were used to compare the significant differences of the mean values reported in Tables 1–6, and the statistical significance of the differences between samples was determined using the *F*-test. The levels of statistical significance were p-values < 0.05 at the 95% confidence level. A correlation analysis of fruit parameters was performed using WinSTAT 2009.1.

3. Results and discussion

3.1. Physicochemical characteristics

The physicochemical characteristics were evaluated for 24 sweet cherry (*P. avium* L.) cultivars, some of which have never been studied before (Donnantonio, Ducignola Nera, Gabbaladri, Genovese, Maiolina Grappolo, Maredda, Minnulara, Napoleona Forestiera, Napoleona Grappolo, Napoleona Verifica, Puntalazzese, Toscana and Zio Peppino). The results are reported in Table 1. Fruit weight, in most cherry cultivars, depends on crop load (Gonçalves et al., 2006; Usenik et al., 2008) and fruit maturity stage (Drake & Elfving, 2002; Serrano et al., 2009; Usenik et al., 2010). The average fruit weight ranged from 3.85 (Ducignola Nera) to 12.97 g (Early Star). Our results show a similar fruit weight for Burlat as that found by Faniadis et al. (2010) and a lower fruit weight in Sweet Heart with respect to the results reported by Girard and Kopp (1998). Fruit firmness varied from 3.25 (Moreau) to 27.00 N (Minnulara). High values of firmness were also found in the Puntalazzese (25.43), Toscana (23.63), Gabbaladri (23.90) and Genovese (18.40) cultivars. Since cherry fruits possess negligible polygalacturonase activity, the different values of firmness found for the

Table 1
Physicochemical characteristics of different sweet cherry cultivars[a].

Cherry cultivar	Fruit weight (g)	Firmness (N)	pH	SSC (°Brix)	TA (% malic acid)	SSC/TA
Black Star	9.02 ± 1.05 q	9.92 ± 1.30 h	3.72 ± 0.01 a	22.73 ± 0.20 v	1.34 ± 0.02 o	17.01 ± 0.25 c
Blaze Star	8.84 ± 0.39 p	13.05 ± 2.98 n	4.01 ± 0.02 f	20.27 ± 0.30 o	1.00 ± 0.01 m	20.22 ± 0.40 j
Burlat	9.84 ± 0.29 t	6.80 ± 2.58 c	4.21 ± 0.01 jk	18.37 ± 0.27 i	0.83 ± 0.03 h	22.23 ± 0.30 o
Donnantonio	8.13 ± 0.33 n	9.53 ± 5.41 g	4.36 ± 0.03 n	20.68 ± 0.31 p	0.75 ± 0.02 d	27.57 ± 0.32 t
Ducignola Nera	3.85 ± 0.05 a	12.86 ± 4.71 m	4.22 ± 0.02 jk	22.52 ± 0.22 u	1.01 ± 0.01 m	22.24 ± 0.43 o
Early Star	12.97 ± 0.32 w	8.47 ± 4.00 f	4.00 ± 0.02 ef	15.22 ± 0.31 c	1.02 ± 0.04 mn	15.00 ± 0.39 a
Ferrovia	8.24 ± 0.06 o	17.13 ± 3.95 q	3.98 ± 0.03 e	19.71 ± 0.19 n	0.89 ± 0.02 j	22.06 ± 0.31 m
Gabbaladri	5.12 ± 0.01 e	23.90 ± 4.06 u	4.26 ± 0.01 m	21.48 ± 0.20 r	0.68 ± 0.01 b	31.48 ± 0.61 u
Genovese	6.37 ± 0.42 i	18.40 ± 7.40 s	4.25 ± 0.02 lm	18.42 ± 0.26 j	0.78 ± 0.03 ef	23.68 ± 0.45 p
Giorgia	9.62 ± 0.36 s	11.53 ± 3.78 k	3.89 ± 0.04 bc	18.87 ± 0.27 k	1.04 ± 0.01 n	18.17 ± 0.33 f
Grace Star	7.09 ± 0.26 k	16.30 ± 2.57 p	3.91 ± 0.02 c	17.21 ± 0.21 f	0.94 ± 0.05 l	18.30 ± 0.38 g
Maiolina Grappolo	4.33 ± 0.04 b	13.06 ± 1.10 n	4.27 ± 0.02 m	16.56 ± 0.20 e	0.68 ± 0.04 b	24.53 ± 0.46 r
Maredda	4.65 ± 0.40 c	7.93 ± 1.82 e	4.18 ± 0.01 i	19.40 ± 0.30 l	0.81 ± 0.01 gh	24.06 ± 0.32 q
Minnulara	4.78 ± 0.15 d	27.00 ± 9.26 w	4.81 ± 0.02 p	21.77 ± 0.33 s	0.57 ± 0.02 a	38.19 ± 0.46 v
Moreau	8.05 ± 0.38 m	3.25 ± 2.00 a	4.23 ± 0.03 kl	17.56 ± 0.31 h	0.79 ± 0.02 fg	22.22 ± 0.38 o
Napoleona Forestiera	9.21 ± 0.70 r	12.41 ± 3.11 l	4.35 ± 0.04 n	17.29 ± 0.25 g	0.86 ± 0.03 i	20.10 ± 0.36 i
Napoleona Grappolo	3.87 ± 0.01 a	10.10 ± 3.40 j	4.62 ± 0.02 o	15.21 ± 0.24 c	0.56 ± 0.02 a	27.41 ± 0.61 s
Napoleona Verifica	6.26 ± 0.55 h	13.37 ± 5.00 o	4.13 ± 0.01 h	16.44 ± 0.24 d	0.93 ± 0.01 kl	17.63 ± 0.66 d
Puntalazzese	6.78 ± 0.20 j	25.43 ± 6.24 v	4.05 ± 0.03 g	22.31 ± 0.28 t	1.35 ± 0.05 o	16.71 ± 0.51 b
Sunburst	11.19 ± 0.03 u	10.07 ± 4.22 i	3.95 ± 0.01 d	19.73 ± 0.26 n	0.91 ± 0.01 jk	21.70 ± 0.58 l
Sweet Early	11.59 ± 0.58 v	6.51 ± 4.37 b	3.87 ± 0.02 b	13.53 ± 0.36 a	0.76 ± 0.01 de	17.80 ± 0.33 e
Sweet Heart	7.87 ± 0.29 l	17.65 ± 2.21 r	3.94 ± 0.01 d	20.78 ± 0.34 q	0.94 ± 0.02 l	22.17 ± 0.56 n
Toscana	5.67 ± 0.45 g	23.63 ± 6.98 t	4.20 ± 0.03 ij	19.55 ± 0.37 m	0.93 ± 0.01 kl	20.91 ± 0.49 k
Zio Peppino	5.58 ± 0.84 f	7.60 ± 1.54 d	4.14 ± 0.03 h	13.66 ± 0.32 b	0.72 ± 0.03 c	19.10 ± 0.43 h

Mean values with different letters (a–w) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

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Table 2
Colour characteristics of different sweet cherry cultivars[a].

Cherry cultivar	L^*	a^*	b^*	C	h
Black Star	24.75 ± 0.80 a	4.81 ± 2.00 d	0.92 ± 0.58 b	4.90 ± 2.06 d	9.74 ± 0.06 d
Blaze Star	25.23 ± 1.04 c	5.95 ± 3.28 e	1.64 ± 1.02 f	6.18 ± 3.43 e	14.90 ± 0.04 i
Burlat	25.89 ± 0.87 g	4.44 ± 1.80 b	1.13 ± 0.62 d	4.58 ± 1.89 b	13.18 ± 0.07 f
Donnantonio	26.83 ± 0.57 l	6.86 ± 2.26 g	1.90 ± 0.82 h	7.12 ± 2.39 g	14.90 ± 0.04 i
Ducignola Nera	31.33 ± 0.87 u	12.08 ± 3.22 p	3.27 ± 1.19 p	12.52 ± 3.42 o	14.90 ± 0.03 i
Early Star	25.48 ± 0.70 e	6.99 ± 2.89 h	2.22 ± 0.94 k	7.33 ± 3.04 h	17.76 ± 0.02 l
Ferrovia	28.39 ± 1.57 r	14.62 ± 6.51 s	4.69 ± 2.57 s	15.36 ± 6.98 r	17.19 ± 0.04 k
Gabbaladri	39.17 ± 5.93 w	30.26 ± 3.91 x	15.42 ± 4.24 x	34.10 ± 4.73 w	26.93 ± 0.10 o
Genovese	26.50 ± 1.05 i	13.36 ± 4.85 r	3.97 ± 2.02 r	13.95 ± 5.22 q	16.04 ± 0.04 j
Giorgia	26.60 ± 1.05 j	9.67 ± 2.95 m	2.99 ± 1.00 n	10.13 ± 3.10 m	17.19 ± 0.03 k
Grace Star	25.36 ± 1.56 d	15.51 ± 4.76 t	5.08 ± 1.72 t	16.32 ± 5.06 s	17.76 ± 0.02 l
Maiolina Grappolo	26.16 ± 1.43 h	8.44 ± 5.58 k	1.84 ± 1.08 g	8.66 ± 5.83 k	9.17 ± 0.10 c
Maredda	25.17 ± 1.02 b	6.54 ± 4.79 f	1.37 ± 1.08 e	6.71 ± 5.04 f	8.59 ± 0.10 b
Minnulara	27.16 ± 0.82 n	8.05 ± 2.16 j	1.99 ± 0.76 i	8.30 ± 2.26 j	13.75 ± 0.04 g
Moreau	26.50 ± 0.99 i	2.37 ± 0.98 a	0.42 ± 0.12 a	2.41 ± 0.95 a	0.57 ± 0.25 a
Napoleona Forestiera	26.71 ± 1.40 k	7.82 ± 4.93 i	2.10 ± 1.79 j	8.11 ± 5.22 i	13.18 ± 0.08 f
Napoleona Grappolo	27.10 ± 1.87 m	13.14 ± 5.16 q	3.65 ± 1.93 q	13.64 ± 5.48 p	14.90 ± 0.03 i
Napoleona Verifica	31.39 ± 2.03 v	22.97 ± 6.33 w	8.33 ± 3.04 w	24.45 ± 6.97 v	19.48 ± 0.04 n
Puntalazzese	27.47 ± 1.24 o	9.75 ± 5.00 n	2.61 ± 1.87 m	10.11 ± 5.32 m	14.32 ± 0.04 h
Sunburst	28.95 ± 1.44 s	17.87 ± 4.31 u	5.94 ± 1.79 u	18.84 ± 4.64 t	18.33 ± 0.02 m
Sweet Early	25.67 ± 0.68 f	4.66 ± 1.83 c	1.03 ± 0.53 c	4.78 ± 1.89 c	12.03 ± 0.05 e
Sweet Heart	31.19 ± 1.78 t	18.35 ± 6.14 v	6.09 ± 2.75 v	19.35 ± 6.70 u	17.76 ± 0.04 l
Toscana	27.64 ± 0.98 p	8.57 ± 4.53 l	2.24 ± 1.67 l	8.87 ± 4.80 l	13.18 ± 0.06 f
Zio Peppino	27.98 ± 0.90 q	11.52 ± 3.10 o	3.11 ± 1.05 o	11.94 ± 3.26 n	14.90 ± 0.02 i

Mean values with different letters (a–x) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

Table 3
Sugar (g/100 g FW) and organic acid contents (mg/100 g FW) of different sweet cherry cultivars[a].

Cherry cultivar	Glucose	Fructose	Sorbitol	Malic acid	Shikimic acid	Fumaric acid
Black Star	8.88 ± 0.24 t	7.06 ± 0.05 q	3.66 ± 0.07 n	1274.60 ± 1.41 v	2.34 ± 0.01 i	0.16 ± 0.01 b
Blaze Star	8.91 ± 1.20 u	7.29 ± 1.15 s	3.91 ± 1.29 p	928.62 ± 1.84s	1.57 ± 0.02 c	0.27 ± 0.01 d
Burlat	7.90 ± 0.18 k	6.73 ± 0.03 n	1.41 ± 0.12 d	750.33 ± 1.36 g	2.02 ± 0.01 g	0.41 ± 0.02 hij
Donnantonio	7.94 ± 0.04 l	6.84 ± 0.05 p	3.65 ± 0.08 n	773.41 ± 1.34 h	3.49 ± 0.01 o	0.39 ± 0.01 g
Ducignola Nera	8.92 ± 0.04 u	7.08 ± 0.11 q	4.46 ± 0.01 q	1276.84 ± 2.81 w	4.38 ± 0.01 r	0.39 ± 0.01 g
Early Star	6.04 ± 0.14 d	4.96 ± 0.04 d	1.32 ± 0.04 c	837.16 ± 4.19 m	1.53 ± 0.01 b	0.36 ± 0.01 f
Ferrovia	8.32 ± 0.15 n	7.07 ± 0.09 q	2.98 ± 0.16 j	898.50 ± 2.11 q	1.73 ± 0.01 d	0.30 ± 0.01 e
Gabbaladri	8.80 ± 0.17 s	7.57 ± 0.09 t	2.97 ± 0.05 j	798.44 ± 2.88 k	2.78 ± 0.05 m	0.48 ± 0.09 l
Genovese	7.15 ± 0.07 g	6.09 ± 0.01 h	3.01 ± 0.04 k	714.52 ± 5.78 d	2.68 ± 0.06 l	0.42 ± 0.14 ij
Giorgia	8.09 ± 0.22 m	6.60 ± 0.25 l	2.51 ± 0.18 h	1043.00 ± 2.08 t	1.75 ± 0.01d	0.15 ± 0.02 b
Grace Star	7.20 ± 0.08 h	5.58 ± 0.08 f	2.01 ± 0.13 f	906.81 ± 3.93 r	1.97 ± 0.03 f	0.19 ± 0.01 c
Maiolina Grappolo	6.43 ± 0.08 f	5.68 ± 0.05 g	1.29 ± 0.06 b	727.23 ± 10.45 f	4.01 ± 0.12 q	1.14 ± 0.29 p
Maredda	7.48 ± 0.14 j	6.58 ± 0.05 l	3.89 ± 0.07 p	823.29 ± 1.09 l	3.31 ± 0.02 n	0.85 ± 0.03 n
Minnulara	8.64 ± 0.07 q	7.30 ± 0.11 s	3.45 ± 0.03 m	725.43 ± 4.16 e	4.46 ± 0.04 s	0.72 ± 0.03 m
Moreau	1.72 ± 0.09 a	6.49 ± 0.11 j	6.77 ± 0.08 r	797.57 ± 1.86 j	1.85 ± 0.01 e	0.49 ± 0.01 l
Napoleona Forestiera	7.40 ± 0.18 i	6.27 ± 0.11 i	1.53 ± 0.11 e	774.97 ± 2.11 i	2.52 ± 0.02 j	0.43 ± 0.01 jk
Napoleona Grappolo	5.50 ± 0.05 b	4.84 ± 0.09 c	0.94 ± 0.12 a	399.97 ± 2.00 a	2.66 ± 0.02 l	0.45 ± 0.04 k
Napoleona Verifica	6.38 ± 0.30 e	5.53 ± 0.10 e	1.53 ± 0.08 e	893.30 ± 0.42 p	2.54 ± 0.01 j	0.50 ± 0.01 l
Puntalazzese	8.31 ± 0.08 n	7.18 ± 0.08 r	3.73 ± 0.04 o	1405.88 ± 8.12 x	3.29 ± 0.02 n	0.41 ± 0.01 hij
Sunburst	8.36 ± 0.08 o	6.78 ± 0.07 o	2.79 ± 0.10 i	871.65 ± 0.40 n	1.74 ± 0.01 d	0.12 ± 0.01 a
Sweet Early	5.98 ± 0.18 c	4.69 ± 0.12 a	1.32 ± 0.10 c	707.09 ± 0.74 c	1.47 ± 0.01 a	0.14 ± 0.01 ab
Sweet Heart	8.77 ± 0.23 r	6.65 ± 0.06 m	3.33 ± 0.03 l	878.91 ± 1.36 o	2.60 ± 0.01 k	0.19 ± 0.03 c
Toscana	8.40 ± 0.08 p	6.54 ± 0.09 k	2.39 ± 0.08 g	1176.62 ± 0.98 u	3.67 ± 0.01 p	0.40 ± 0.01 hi
Zio Peppino	5.97 ± 0.06 c	4.77 ± 0.09 b	0.93 ± 0.09 a	633.17 ± 16.18 b	2.16 ± 0.12 h	0.93 ± 0.15 o

Mean values with different letters (a–x) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

diverse genotypes could be due to the composition of their cell walls (Batisse, Buret, & Coulomb, 1996). The pH values ranged from 3.72 (Black Star) to 4.62 (Napoleona Grappolo). These values are in accordance with other data reported in literature (Girard & Kopp, 1998). As already observed by Girard and Kopp (1998), the highest correlation obtained was that of pH *vs* titratable acidity (TA) with a Pearson's correlation coefficient $r = -0.706$ (p-value < 0.05; $n = 72$). A wide range of SSC and TA was found among the cherry cultivars, as indicated by a lower correlation coefficient value ($r = 0.425$; p-value < 0.05; $n = 72$). The SSC varied from 13.53 (Sweet Early) to 22.73 °Brix (Black Star), while the values of TA ranged from 0.57 (Minnulara) to 1.35% of malic acid (Puntalazzese). An accumulation of acidity was observed in different sweet cherry cultivars as the harvesting date was delayed by Díaz-Mula et al. (2009). Therefore, the high values of TA and SSC found in the Black Star and Puntalazzese cultivars indicate an advanced stage of fruit ripening. It is well known that the flavour intensity of a sweet cherry, which is mainly linked to the SSC/TA ratio (Crisosto, Crisosto, & Metheney, 2003), appears to be a key factor influencing preference and acceptability by consumers. In this study we found that the

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Table 4
Phenolic content (mg/100 g FW) of different sweet cherry cultivars[a].

Cherry cultivar	Neochlorogenic acid	*p*-Coumaroylquinic acid	Chlorogenic acid
Black Star	47.52 ± 1.38 v	6.01 ± 1.96 j	1.17 ± 0.70 i
Blaze Star	23.22 ± 3.75 n	26.62 ± 4.39 x	2.33 ± 0.44 s
Burlat	9.18 ± 0.72 c	11.28 ± 0.51 r	1.83 ± 0.14 n
Donnantonio	9.59 ± 0.45 d	5.08 ± 0.29 i	1.26 ± 0.14 j
Ducignola Nera	71.45 ± 2.68 w	7.74 ± 3.09 n	1.93 ± 1.03 o
Early Star	11.69 ± 1.07 i	15.97 ± 1.21 w	2.62 ± 0.16 t
Ferrovia	26.99 ± 8.35 p	6.39 ± 2.18 k	1.48 ± 0.66 kl
Gabbaladri	45.33 ± 1.25 u	1.97 ± 0.57 d	0.61 ± 0.41 g
Genovese	19.50 ± 0.42 l	1.62 ± 0.04 c	0.17 ± 0.02 a
Giorgia	25.27 ± 0.33 o	3.35 ± 0.08 g	0.42 ± 0.08 e
Grace Star	29.59 ± 0.32 q	3.48 ± 0.02 h	0.46 ± 0.02 f
Maiolina Grappolo	32.51 ± 0.59 r	2.65 ± 0.08 e	0.42 ± 0.05 e
Maredda	74.09 ± 9.25 x	6.78 ± 0.80 m	0.74 ± 0.55 h
Minnulara	22.56 ± 0.64 m	1.01 ± 0.02 b	0.28 ± 0.01 c
Moreau	13.39 ± 1.39 j	15.81 ± 2.10 v	1.99 ± 0.28 p
Napoleona Forestiera	10.47 ± 1.59 f	15.53 ± 3.98 t	2.12 ± 0.55 r
Napoleona Grappolo	11.45 ± 0.35 g	9.45 ± 0.13 q	1.82 ± 0.10 n
Napoleona Verifica	11.60 ± 0.15 h	12.61 ± 1.78 t	2.07 ± 0.24 q
Puntalazzese	34.66 ± 2.02 s	0.95 ± 0.05 a	0.21 ± 0.01 b
Sunburst	16.92 ± 0.30 k	9.25 ± 0.23 p	1.62 ± 0.03 m
Sweet Early	6.27 ± 0.48 a	8.40 ± 0.70 o	1.49 ± 0.10 l
Sweet Heart	9.88 ± 0.51 e	6.44 ± 0.23 l	1.46 ± 0.10 k
Toscana	35.30 ± 1.18 t	3.13 ± 0.13 f	0.33 ± 0.07 d
Zio Peppino	7.91 ± 1.98 b	12.34 ± 2.98 s	2.97 ± 0.61 u

Mean values with different letters (a–x) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

Gabbaladri and Minnulara cultivars had significantly higher (p-value < 0.05) SSC/TA ratios (31.48 and 38.19, respectively) compared to those of Early Star and Puntalazzese, which had the lowest values (15.00 and 16.71, respectively). Therefore, it can be assumed that the taste of the Gabbaladri and Minnulara fruits was sweeter than that of the Early Star and Puntalazzese.

The colour characteristics of the sweet cherry cultivars are reported in Table 2. Skin colour is considered to be the most important index of cherry quality and maturity (Gao & Mazza, 1995). Some of the factors influencing the colour of cherries are the concentration and distribution of the different anthocyanins and colourless phenolics as well as the pH (Gao & Mazza, 1995; Girard & Kopp, 1998). The colour of fruits can be enhanced by co-pigmentation, a phenomenon in which organic compounds such as flavonoids or phenolics bind through weak hydrophobic bonds to the anthocyanins. On the other hand, as pH increases, the colour of anthocyanins moves to the non-spectral purple and approaches a progressive loss of fruit colour (Gonçalves et al., 2007).

The result of this study showed that L^* values ranged from 24.75 (Black Star) to 39.17 (Gabbaladri). In addition, darker cherries such as Black Star and Moreau (lower L^* values) tended to be less red (lower a^* values) and less yellow (lower b^* values). Cultivars Gabbaladri and Napoleona Verifica were lighter, redder and yellower than the others. Furthermore, these cultivars showed higher values of chroma (C) and hue angle (h), indicators of a lighter and less intense red colour, while Black Star and Moreau had lower C and h values, corresponding to a darker and more intense colour.

The sugar and organic acid contents of the sweet cherry cultivars are shown in Table 3. Trace amounts of sucrose were also identified in most of the genotypes analysed but have not been included in Table 3. Monosaccharides such as glucose and fructose are predominant in sweet cherry fruits, together accounting for more than 80% of the total sugar content. According to literature (Girard & Kopp, 1998; Usenik et al., 2008, 2010), glucose was found in the highest content, followed by fructose and sorbitol. The highest sugar level was found in Ducignola Nera (20.46 g/100 g FW) while the lowest was in Napoleona Grappolo (11.28 g/100 g FW). Cultivar Blaze Star had the highest glucose content and Moreau the lowest (8.91 and 1.72 g/100 g FW, respectively). The content of fructose varied from 4.69 to 7.57 g/100 g FW for cultivars Sweet Early and Gabbaladri, respectively. The genotypes with a higher glucose content also had a higher fructose level, similar to the tendency described for sour cherries (Papp et al., 2010). Finally, the total content of sugars was highly correlated with SSC (r = 0.966; p-value < 0.05; n = 72). Sweet cherries accumulate large

Table 5
Anthocyanin content (mg CGE/100 g FW) and relative amounts (% of the total anthocyanins) of different sweet cherry cultivars[a].

Cherry cultivar	Cyanidin 3-glucoside		Cyanidin 3-rutinoside		Pelargonidin 3-rutinoside		Peonidin 3-rutinoside	
	mg CGE/100 g FW	%	mg CGE/100 g FW	%	mg CGE/100 g FW	%	mg CGE/100 g FW	%
Black Star	6.97 ± 0.16 j	8.14 ± 0.19 ij	68.28 ± 0.10 u	79.76 ± 0.10 h	2.01 ± 0.03 j	2.35 ± 0.03 hi	8.35 ± 0.11 s	9.75 ± 0.12 o
Blaze Star	2.71 ± 0.03 g	4.34 ± 0.05 abc	51.87 ± 0.14 s	83.16 ± 0.20 i	3.06 ± 0.10 l	4.91 ± 0.16 m	4.73 ± 0.01 r	7.59 ± 0.01 n
Burlat	34.84 ± 0.44 q	41.19 ± 0.53 p	46.92 ± 0.43 q	55.48 ± 0.50 b	0.50 ± 0.01 g	0.59 ± 0.01 a	2.31 ± 0.03 m	2.73 ± 0.03 gh
Donnantonio	1.40 ± 0.07 d	4.27 ± 0.20 ab	29.64 ± 0.01 m	90.46 ± 0.02 n	0.47 ± 0.07 fg	1.44 ± 0.21 cde	1.25 ± 0.01 j	3.83 ± 0.01 j
Ducignola Nera	3.21 ± 0.16 h	8.01 ± 0.39 ij	35.67 ± 0.17 p	88.93 ± 0.40 m	0.47 ± 0.02 efg	1.17 ± 0.05 abcde	0.76 ± 0.02 g	1.89 ± 0.04 de
Early Star	13.42 ± 0.14 n	28.42 ± 0.31 n	32.11 ± 0.15 n	67.99 ± 0.30 d	0.28 ± 0.01 bcdef	0.60 ± 0.01 a	1.41 ± 0.01 k	2.99 ± 0.01 h
Ferrovia	2.08 ± 0.01 f	7.72 ± 0.03 hij	22.88 ± 0.03 i	84.74 ± 0.05 j	0.39 ± 0.01 defg	1.44 ± 0.01 cde	1.65 ± 0.01 l	6.10 ± 0.02 m
Gabbaladri	0.40 ± 0.01 a	6.47 ± 0.08 fg	5.69 ± 0.02 a	91.90 ± 0.18 o	0.09 ± 0.01 ab	1.39 ± 0.09 bcde	0.01 ± 0.01 a	0.24 ± 0.01 a
Genovese	1.71 ± 0.01 e	6.80 ± 0.06 fgh	22.68 ± 0.01 hi	90.44 ± 0.03 n	0.27 ± 0.03 abcd	1.06 ± 0.14 abcde	0.43 ± 0.01 de	1.70 ± 0.05 cde
Giorgia	2.72 ± 0.02 g	8.36 ± 0.07 j	25.20 ± 0.11 j	77.55 ± 0.30 g	1.29 ± 0.05 i	3.98 ± 0.16 l	3.28 ± 0.07 o	10.11 ± 0.22 p
Grace Star	1.43 ± 0.03 d	5.25 ± 0.10 de	22.57 ± 0.09 g	82.99 ± 0.30 i	0.25 ± 0.01 abcd	0.92 ± 0.04 abcd	1.41 ± 0.10 n	2.99 ± 0.36 q
Maiolina Grappolo	9.67 ± 0.11 l	26.07 ± 0.03 m	26.17 ± 0.01 l	70.58 ± 0.01 e	0.27 ± 0.01 abcde	0.72 ± 0.03 ab	0.98 ± 0.01 h	2.63 ± 0.01 f
Maredda	23.75 ± 0.07 o	25.22 ± 0.08 m	67.82 ± 0.10 t	72.00 ± 0.12 f	0.97 ± 0.17 h	1.03 ± 0.18 abcde	1.65 ± 0.02 l	1.75 ± 0.02 de
Minnulara	1.04 ± 0.01 c	3.74 ± 0.04 a	25.99 ± 0.01 l	93.06 ± 0.07 p	0.43 ± 0.02 defg	1.53 ± 0.07 def	0.46 ± 0.01 e	1.66 ± 0.05 cd
Moreau	10.97 ± 0.05 m	34.82 ± 0.17 o	18.61 ± 0.09 e	59.08 ± 0.26 c	0.85 ± 0.05 h	2.69 ± 0.16 ij	1.08 ± 0.02 i	3.41 ± 0.07 i
Napoleona Forestiera	6.48 ± 0.01 i	48.72 ± 0.05 r	6.52 ± 0.01 b	49.05 ± 0.14 a	0.10 ± 0.03 ab	0.77 ± 0.19 abc	0.19 ± 0.01 b	1.46 ± 0.01 bc
Napoleona Grappolo	0.94 ± 0.01 c	6.55 ± 0.04 fg	12.41 ± 0.15 d	86.72 ± 1.16 kl	0.32 ± 0.18 cdefg	2.26 ± 1.25 ghi	0.64 ± 0.01 f	4.47 ± 0.06 k
Napoleona Verifica	0.80 ± 0.01 bc	7.62 ± 0.02 hij	8.95 ± 0.02 c	85.65 ± 0.11 jk	0.08 ± 0.01 a	0.81 ± 0.01 abc	0.62 ± 0.01 f	5.93 ± 0.08 lm
Puntalazzese	8.16 ± 0.21 k	13.99 ± 0.36 l	48.26 ± 0.03 r	82.72 ± 0.07 i	0.79 ± 0.25 h	1.35 ± 0.43 bcde	1.13 ± 0.01 i	1.94 ± 0.01 e
Sunburst	1.46 ± 0.02 de	5.92 ± 0.08 ef	21.84 ± 0.14 f	88.59 ± 0.62 m	0.41 ± 0.18 defg	1.66 ± 0.74 efgh	0.94 ± 0.01 h	3.82 ± 0.04 j
Sweet Early	5.54 ± 0.85 p	15.63 ± 4.79 q	24.34 ± 2.60 o	76.68 ± 4.62 a	0.26 ± 0.01 k	0.82 ± 0.17 k	2.00 ± 1.44 q	6.86 ± 0.99 m
Sweet Heart	1.39 ± 0.02 d	4.95 ± 0.07 cde	22.42 ± 0.07 g	79.82 ± 0.22 h	0.44 ± 0.02 defg	1.58 ± 0.07 defg	3.83 ± 0.06 p	13.64 ± 0.22 r
Toscana	2.85 ± 0.09 g	9.81 ± 0.32 k	25.51 ± 0.04 k	87.87 ± 0.11 lm	0.32 ± 0.01 cdefg	1.11 ± 0.01 abcde	0.35 ± 0.06 c	1.22 ± 0.20 b
Zio Peppino	0.55 ± 0.16 ab	7.32 ± 2.10 ghi	6.40 ± 0.18 b	84.80 ± 2.23 j	0.16 ± 0.03 abc	2.17 ± 0.39 fghi	0.43 ± 0.02 de	5.71 ± 0.27 l

Mean values with different letters (a–u) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

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Table 6
Total anthocyanin contents (mg CGE/100 g FW), total phenol contents (mg GAE/100 g FW) and antioxidant activities (μmol TE/100 g FW) of different sweet cherry cultivars[a].

Cherry cultivar	Total anthocyanins	Total phenols	Antioxidant activity
Black Star	85.61 ± 1.14 w	145.69 ± 7.71 s	2226.00 ± 9.89 n
Blaze Star	62.38 ± 0.39 t	138.80 ± 3.01 r	2395.00 ± 14.84 p
Burlat	84.59 ± 1.55 v	122.04 ± 1.26 n	2452.50 ± 6.57 r
Donnantonio	32.77 ± 3.06 o	103.79 ± 7.51 h	2966.00 ± 10.72 v
Ducignola Nera	40.11 ± 0.44 q	149.45 ± 6.09 u	1307.75 ± 6.37 g
Early Star	47.24 ± 2.17 r	87.00 ± 4.37 c	1306.00 ± 5.94 f
Ferrovia	27.01 ± 3.96 h	97.40 ± 4.96 f	962.00 ± 22.49 d
Gabbaladri	6.21 ± 1.73 a	105.30 ± 4.15 j	2013.50 ± 30.90 k
Genovese	25.08 ± 0.47 g	105.05 ± 7.48 i	1703.50 ± 6.36 i
Giorgia	32.50 ± 4.58 n	115.05 ± 0.06 m	2050.50 ± 17.32 l
Grace Star	27.21 ± 1.81 i	114.60 ± 2.20 l	2226.00 ± 9.89 n
Maiolina Grappolo	37.09 ± 1.61 p	145.99 ± 8.97 t	1010.50 ± 3.60 e
Maredda	94.20 ± 2.10 x	162.21 ± 0.36 x	2394.50 ± 27.93 o
Minnulara	27.94 ± 1.71 j	124.12 ± 0.32 p	2130.50 ± 19.87 m
Moreau	31.51 ± 2.25 m	154.47 ± 2.82 v	3166.00 ± 9.96 w
Napoleona Forestiera	13.31 ± 2.41 d	123.60 ± 4.37 o	2677.50 ± 4.87 t
Napoleona Grappolo	14.32 ± 0.05 e	84.96 ± 3.37 a	646.00 ± 7.77 a
Napoleona Verifica	10.47 ± 3.34 c	89.84 ± 0.94 e	808.50 ± 17.68 c
Puntalazzese	58.35 ± 1.79 s	156.00 ± 1.30 w	2688.50 ± 29.34 u
Sunburst	24.66 ± 0.23 f	102.76 ± 1.91 g	2571.00 ± 28.00 s
Sweet Early	70.65 ± 1.27 u	110.79 ± 2.01 k	2404.00 ± 5.94 q
Sweet Heart	28.10 ± 1.17 k	89.56 ± 0.16 d	728.00 ± 21.21 b
Toscana	29.04 ± 1.71 l	131.66 ± 3.72 q	1393.75 ± 7.15 h
Zio Peppino	7.56 ± 0.23 b	86.08 ± 2.04 b	1797.50 ± 19.16 j

Mean values with different letters (a–x) within the same column are statistically different (p-value < 0.05).
[a] Values are expressed as the mean ± standard deviation.

quantities of sorbitol, as do other fruits such as apples, pears, peaches, and prunes (Noiraud, Maurousset, & Lemoine, 2001). Sorbitol is a sugar alcohol that contributes to the beneficial health effects of cherry fruits including diet control and dental health (Kelebek & Selli, 2011). The level of this component in the different genotypes ranged from 0.93 to 6.77 g/100 g FW for Zio Peppino and Moreau cultivars, respectively. The latter variety contains a low content of glucose and a very high value of sorbitol, almost double than that of other cultivars studied. Sorbitol is synthesised in the leaf from glucose-6-phopsphate (G6P) by sorbitol-6-phosphate dehydrogenase enzyme (S6PDH) and afterwards is translocated to the fruit (Teo et al., 2006). A previous study carried out on pear leaf tissue demonstrated that sorbitol was poorly metabolised while glucose was largely converted to sorbitol (Bieleski, 1977). Therefore, the high level of sorbitol in a Moreau cherry can be explained by the metabolism that occurs in this cultivar.

Malic acid, shikimic acid and fumaric acid were detected in the different cherry cultivars (Table 3). Malic acid was the predominant organic acid, accounting for more than 98% of the total organic acid content. Shikimic acid and fumaric acid were minor constituents (0.30% and 0.05% of the total, respectively). The highest sum of organic acids was found in Puntalazzese (1409.58 mg/100 g FW) and the lowest in Napoleona Grappolo (403.07 mg/100 g FW). The content of individual organic acids differed widely among the cultivars: malic acid ranged from 399.97 to 1276.60 mg/100 g FW (Napoleona Grappolo, Puntalazzese), shikimic acid ranged from 1.47 to 4.46 mg/100 g FW (Sweet Early, Minnulara) and fumaric acid ranged from 0.12 to 0.93 mg/100 g FW (Sunburst, Zio Peppino). The different content of organic acids was reflected in the TA levels. In fact, Black Star, Ducignola Nera, Giorgia and Puntalazzese cultivars that had the highest levels of malic acid showed TA values higher than 1%. Additionally, a high correlation was observed between the total content of organic acids and TA levels ($r = 0.873$; p-value < 0.05; $n = 72$).

3.2. Phenolic compounds

The phenolic compound content (without anthocyanins) of sweet cherry cultivars is shown in Table 4. Neochlorogenic acid, *p*-coumaroylquinic acid and chlorogenic acid were detected and quantified in the sweet cherry cultivars by HPLC-PDA analysis (Fig. 1). The same phenolic compounds were present in each cultivar, but there were differences in the relative levels. In almost all genotypes, neochlorogenic acid was the major hydroxycinnamic acid derivative followed by *p*-coumaroylquinic acid and chlorogenic acid. The highest concentration of phenolic compounds was found in Maredda (81.62 mg/100 g FW) and the lowest in Donnantonio (15.93 mg/100 g FW). Regarding the neochlorogenic acid levels, the different genotypes can be classified into three groups: the first group with neochologenic acid levels ranging from 40 to 75 mg/100 g FW (Gabbaladri, Black Star, Ducignola Nera and Maredda); the second group with intermediate neochologenic acid levels between 20 and 40 mg/100 g FW (Genovese, Minnulara, Blaze Star, Giorgia, Ferrovia, Grace Star, Maiolina Grappolo, Puntalazzese and Toscana); and the third group with much lower neochologenic acid levels ranging from 6 to 20 mg/100 g FW (Sweet Early, Zio Peppino, Burlat, Donnantonio, Sweet Heart, Napoleona Forestiera, Napoleona Grappolo, Napoleona Verifica, Early Star, Moreau and Sunburst). In some sweet cherry varieties, such as: Blaze Star, Burlat, Early Star, Moreau, Napoleona Forestiera, Sweet Early and Zio Peppino, the levels of *p*-coumaroylquinic acid were found to be higher than that of neochlorogenic acid. Mozetic et al. (2002), has demonstrated that the levels of hydroxycinnamic derivatives such as neochlorogenic acid and *p*-coumarylquinic acid decrease during ripening. A change in their ratio could also be due to the hydroxylation of *p*-coumarylquinic acid to neochlorogenic acid and both most likely contribute to anthocyanin biosynthesis. In the present study, the concentrations of hydroxycinnamic acid derivatives are higher than the values reported by González-Gómez et al. (2010) and Usenik et al. (2008), while they are lower than the values obtained by Kelebek and Selli (2011) because of the different cultivars analysed and the methods of extraction and determination used, but the proportions among individual phenolics are similar.

The four anthocyanins detected in the studied sweet cherry cultivars were cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-rutinoside and peonidin 3-rutinoside (Fig. 2). These are the same as those identified in different varieties by other authors (González-Gómez et al., 2010; Liu et al., 2011; Mozetic et al., 2002; Usenik et al., 2008). Significant variations (p-value < 0.05) were found in the specific anthocyanin contents between different genotypes (Table 5). The amount of cyanidin 3-rutinoside ranged from 5.69 to 68.28 mg CGE/100 g FW, while the concentrations of cyanidin 3-glucoside, peonidin 3-rutinoside and pelargonidin 3-rutinoside ranged between 0.40 and 34.84, 0.01 and 8.35, and 0.08 and 3.06 mg CGE/100 g FW, respectively. The highest concentrations of cyanidin 3-rutinoside were observed in the Black Star and Maredda cultivars (68.28 and 67.82 mg CGE/100 g FW, respectively), while the highest contents of cyanidin 3-glucoside were found in Burlat and Maredda (34.84 and 23.75 mg CGE/100 g FW, respectively). The cultivar Napoleona Forestiera showed a similar content of the two cyanidin derivatives (6.52 and 6.48 mg CGE/100 g FW of cyanidin 3-rutinoside and cyanidin 3-glucoside, respectively). The highest concentration of pelargonidin 3-rutinoside was found in the Blaze Star cultivar (3.06 mg CGE/100 g FW), whereas the highest level of peonidin 3-rutinoside was observed in the Black Star (8.35 mg CGE/100 g FW).

3.3. Total anthocyanins, total phenols and antioxidant activity

The total anthocyanin content and total phenol content in sweet cherry cultivars are shown in Table 6. Cultivar Maredda had the

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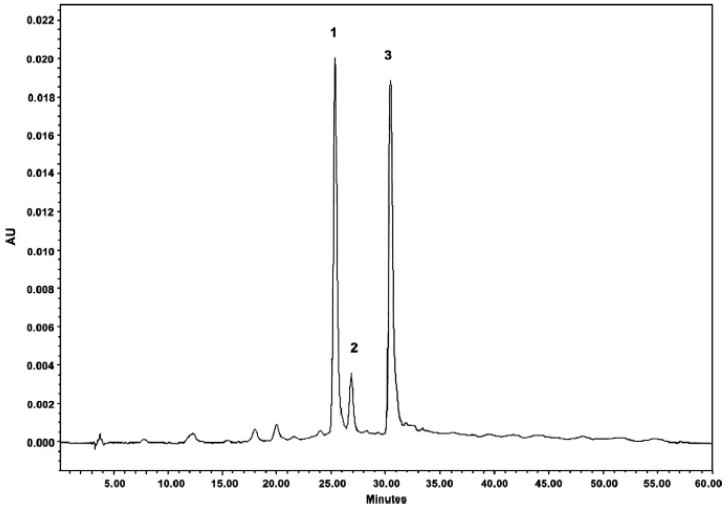

Fig. 1. HPLC chromatogram of the hydroxycinnamic acid derivatives of sweet cherry extract detected at 320 nm. (1) Neochlorogenic acid; (2) chlorogenic acid; (3) *p*-coumaroylquinic acid.

highest total anthocyanin content (94.20 mg CGE/100 g FW), followed by Black Star (85.61 mg CGE/100 g FW), Burlat (84.59 mg CGE/100 g FW) and Sweet Early (70.65 mg CGE/100 g FW). Another group of genotypes showed intermediate values of anthocyanins between 25 and 62 mg CGE/100 g FW (Sunburst, Genovese, Minnulara, Ferrovia, Grace Star, Sweet Heart, Toscana, Moreau, Donnantonio, Maiolina Grappolo, Ducignola Nera, Early Star, Puntalazzese and Blaze Star). Finally, only a few varieties had a content included between 6 and 25 mg CGE/100 g FW (Gabbaladri, Zio Peppino, Napoleona Verifica, Napoleona Forestiera, Napoleona Grappolo and Sunburst). The values of total anthocyanins reported in the present study were in the range of those present in literature (Gao & Mazza, 1995; Gonçalves et al., 2004; Kim et al., 2005).

As observed for total anthocyanin content, a wide range of concentrations of total phenols was noted among the different cherry cultivars tested. The total phenol contents ranged from 84.96 to 162.21 mg GAE/100 g FW for cultivars Napoleona Grappolo and Maredda, respectively (Table 6). The average total phenol content of the 24 sweet cherry cultivars analysed (118.59 mg GAE/100 g FW) resulted in similar values to those measured by Kim et al. (2005) (110 mg GAE/100 g FW). Additionally, Gonçalves et al. (2004) found similar total phenol values for cultivar Burlat (130 mg GAE/100 g FW) with respect to that reported in this study (122.04 mg GAE/100 g FW). The total phenol content was correlated with the sum of the individual phenolic compounds determined by HPLC ($r = 0.644$; p-value < 0.05; $n = 72$).

The antioxidant activities of the sweet cherry fruits, measured by the ORAC assay, are shown in Table 6. The ORAC assay is considered to be a preferable method for the assessment of antioxidant capacity because of its biological relevance to *in vivo* antioxidant efficacy (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). The antioxidant capacity of the sweet cherries was in the range of 646–3166 μmol TE/100 g FW. The lowest values were measured in Napoleona Grappolo and Sweet Heart cultivars (646 and 728 μmol TE/100 g FW, respectively), while the highest values were observed in Donnantonio and Moreau cultivars (2966 and 3166 μmol TE/100 g FW, respectively). The fruits of cultivar Zio Peppino contained low values of total anthocyanins and total phenolics but had a high antioxidant activity (1797.50 μmol TE/100 g FW). As observed by other authors (Kelebek & Selli, 2011; Usenik et al., 2010) the antioxidant activity may also depend on the presence of specific phenolic compounds, such as flavan-3-ols, flavonols and procyanidins, which were not evaluated in this study. Our results were comparable to those observed by Serra et al. (2011), who reported that the antioxidant capacity detected by ORAC assay ranged from 50 to 177 μmol TE/g dry weight (equivalent to 1320–3540 μmol TE/100 g FW). Moreover, a positive correlation ($r = 0.503$; p-value < 0.05; $n = 72$) was observed between ORAC values and total phenol content.

4. Conclusions

In this study, we evaluated the fruit quality parameters and chemical attributes of 24 sweet cherry (*P. avium* L.) cultivars grown in Sicily (Italy). The results of our research showed large variability between cultivars in their physicochemical characteristics. Quantitatively, the major sugar and organic acid compounds in all the

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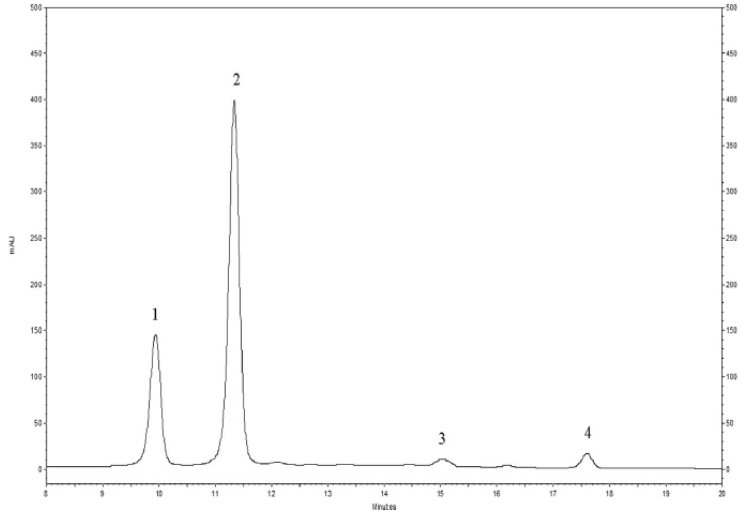

Fig. 2. HPLC chromatogram of the anthocyanins of sweet cherry extract detected at 520 nm. (1) Cyanidin 3-glucoside; (2) cyanidin 3-rutinoside; (3) pelargonidin 3-rutinoside; (4) peonidin 3-rutinoside.

cherry cultivars were found to be glucose and malic acid, respectively. The highest content of total sugars was found in Ducignola Nera, while the lowest was in Napoleona Grappolo. With regard to organic acids, the highest total content was found in Puntalazzese and the lowest in Napoleona Grappolo. Furthermore, we compared the levels of bioactive compounds relevant to human health, such as anthocyanins and phenolics, and evaluated the antioxidant activities of the investigated cherry cultivars using the ORAC assay. Neochlorogenic acid was found to be the main phenolic compound in all of the cherry cultivars, followed by *p*-coumaroylquinic acid and chlorogenic acid. The highest content of phenolic compounds was found in Maredda while the lowest was in Donnantonio. Cyanidin 3-rutinoside was found to be the principal anthocyanin in all of the cherry cultivars, followed by cyanidin 3-glucoside, peonidin 3-rutinoside and pelargonidin 3-rutinoside. The highest content of anthocyanins was found in Maredda, and the lowest was in Gabbaladri. Our results showed that the cultivars had similar qualitative profiles but different amounts of anthocyanins and phenolics. The main phenolic compounds identified were important contributors to the total antioxidant capacity of the tested sweet cherry samples. Additionally, great variations in the contents of both total and individual phenolic compounds as well as antioxidant activities between the different studied genotypes were observed.

Genetic factors may modulate the composition and concentration of phytochemicals. Further investigations are needed to evaluate the effects of the environment and other factors, such as climate, soil characteristics and cultivation techniques, on the nutraceutical properties of cherries in order to develop selection procedures that would be useful for a sweet cherry breeding program in Sicily.

Acknowledgement

We acknowledge "Assessorato Regionale delle Risorse Agricole e Alimentari della Regione Siciliana" for financial support, in the frame of the project "Interventi per il miglioramento produttivo e qualitativo del ciliegio in ambiente etneo (CILLETNA)".

References

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry, 51*, 6657–6662.

Batisse, C., Buret, M., & Coulomb, P. J. (1996). Biochemical differences in cell wall of cherry fruit between soft and crisp fruit. *Journal of Agricultural and Food Chemistry, 44*, 453–457.

Bieleski, R. L. (1977). Accumulation of sorbitol and glucose by leaf slices of *Rosaceae*. *Australian Journal of Plant Physiology, 4*, 11–24.

Crisosto, C. H., Crisosto, G. M., & Metheney, P. (2003). Consumer acceptance of 'Brooks' and 'Bing' cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology, 28*, 159–167.

Díaz-Mula, H. M., Castillo, S., Martínez-Romero, D., Valero, D., Zapata, P. J., Guillén, F., et al. (2009). Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage. *Food Science and Technology International, 15*, 535–543.

Drake, S. R., & Elfving, D. C. (2002). Indicators of maturity and storage quality of 'Lapins' sweet cherry. *HortTechnology, 12*, 687–690.

Esti, M., Cinquanta, L., Sinesio, F., Moneta, E., & Di Matteo, M. (2002). Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage. *Food Chemistry, 76*, 399–405.

Please cite this article in press as: Ballistreri, G., et al. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chemistry* (2012), http://dx.doi.org/10.1016/j.foodchem.2012.11.024

Faniadis, D., Drogoudi, P. D., & Vasilakakis, M. (2010). Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (*Prunus avium* L.). *Scientia Horticulturae, 125*, 301–304.

FAO (Food and Agriculture Organization of the United Nations). (2010). *FAO statistical database*. URL <http://faostat.fao.org> Accessed 30.06.12.

Fazzari, M., Fukumoto, L., Mazza, G., Livrea, M. A., Tesoriere, L., & Di Marco, L. (2008). In vitro bioavailability of phenolic compounds from five cultivars of frozen sweet cherries (*Prunus avium* L.). *Journal of Agricultural and Food Chemistry, 56*, 3561–3568.

Gao, L., & Mazza, G. (1995). Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *Journal of Agricultural and Food Chemistry, 43*, 343–346.

Girard, B., & Kopp, T. G. (1998). Physicochemical characteristics of selected sweet cherry cultivars. *Journal of Agricultural and Food Chemistry, 46*, 471–476.

Gonçalves, B., Landbo, A. K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Rosa, E., et al. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *Journal of Agricultural and Food Chemistry, 52*, 523–530.

Gonçalves, B., Moutinho-Pereira, J., Santos, A., Silva, A. P., Bacelar, E., Correia, C., et al. (2006). Scion–rootstock interaction affects the physiology and fruit quality of sweet cherry. *Tree Physiology, 26*, 93–104.

Gonçalves, B., Silva, A. P., Moutinho-Pereira, J., Bacelar, E., Rosa, E., & Meyer, A. S. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). *Food Chemistry, 103*, 976–984.

González-Gómez, D., Lozano, M., Fernández-León, M. F., Bernalte, M. J., Ayuso, M. C., & Rodríguez, A. B. (2010). Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *Journal of Food Composition and Analysis, 23*, 533–539.

Jacob, R. A., Spinozzi, G. M., Simon, V. A., Kelley, D. S., Prior, R. L., Hess-Pierce, B., et al. (2003). Consumption of cherries lowers plasma urate in healthy women. *Journal of Nutrition, 133*, 1826–1829.

Kang, S.-Y., Seeram, N. P., Nair, M. G., & Bourquin, L. D. (2003). Tart cherry anthocyanins inhibit tumor development in Apc^{Min} mice and reduce proliferation of human colon cancer cells. *Cancer Letters, 194*, 13–19.

Kelebek, H., & Selli, S. (2011). Evaluation of chemical constituents and antioxidant activity of sweet cherry (*Prunus avium* L.) cultivars. *International Journal of Food Science & Technology, 46*, 2530–2537.

Kim, D. O., Heo, H. J., Kim, Y. J., Yang, H. S., & Lee, C. Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. *Journal of Agricultural and Food Chemistry, 53*, 9921–9927.

Liu, Y., Liu, X., Zhong, F., Tian, R., Zhang, K., Zhang, X., et al. (2011). Comparative study of phenolic compounds and antioxidant activity in different species of cherries. *Journal of Food Science, 76*, 633–638.

Mazza, G., & Brouillard, R. (1990). The mechanism of co-pigmentation of anthocyanins in aqueous solutions. *Phytochemistry, 29*, 1097–1102.

Mozetic, B., Trebse, P., & Hribar, J. (2002). Determination and quantitation of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Gorica region (Slovenia). *Food Technology and Biotechnology, 40*, 207–212.

Noiraud, N., Maurousset, L., & Lemoine, R. (2001). Transport of polyols in higher plants. *Plant Physiology and Biochemistry, 39*, 717–728.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4926.

Papp, N., Szilvassy, B., Abranko, L., Szabo, T., Pfeiffer, P., Szabo, S., et al. (2010). Main quality attributes and antioxidants in Hungarian sour cherries: Identification of genotypes with enhanced functional properties. *International Journal of Food Science & Technology, 45*, 395–402.

Rapisarda, P., Fanella, F., & Maccarone, E. (2000). Reliability of analytical methods for determining anthocyanins in blood orange juices. *Journal of Agricultural and Food Chemistry, 48*, 2249–2252.

Seeram, N. P., Momin, R. A., Nair, M. G., & Bourquin, L. D. (2001). Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine, 8*, 362–369.

Serra, A. T., Duarte, R. O., Bronze, M. R., & Duarte, C. M. M. (2011). Identification of bioactive response in traditional cherries from Portugal. *Food Chemistry, 125*, 318–325.

Serrano, M., Díaz-Mula, H. M., Zapata, P. J., Castillo, S., Guillen, F., Martínez-Romero, D., et al. (2009). Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *Journal of Agricultural and Food Chemistry, 57*, 3240–3246.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. *Methods in Enzymology, 299*, 152–178.

Teo, G., Suzuki, Y., Uratsu, S. L., Lampinen, B., Ormonde, N., Hu, W. K., et al. (2006). Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. *Proceedings of the National academy of Sciences of the United States of America, 103*, 18842–18847.

Usenik, V., Fabcic, J., & Stampar, F. (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chemistry, 107*, 185–192.

Usenik, V., Fajt, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., & Veberic, R. (2010). Sweet cherry pomological and biochemical characteristics influenced by rootstock. *Journal of Agricultural and Food Chemistry, 58*, 4928–4933.

Please cite this article in press as: Ballistreri, G., et al. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chemistry* (2012), http://dx.doi.org/10.1016/j.foodchem.2012.11.024

PUBL.3

QUALITY AND FUNCTIONAL PROPERTIES OF CHERRY FRUITS CV "MASTRANTONIO"

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Abstract

The main quality characteristics of "Mastrantonio" sweet cherry harvested at ripening time at three different altitudes 500, 600 and 900 meters were tested.

Quality characteristics (weight, texture, colour, total soluble solids, pH and total acidity), individual sugars, organic acids, bioactive compounds (total phenolics and anthocyanins) and their total antioxidant activity were determined.

Quality characteristics are slightly influenced by the altitudes tested. Colour L*a*b* parameters were higher at 900 mt; lower values of total soluble solids were recorded at 600 mt and data were confirmed by the lower sugars content. Malic and fumaric acids contents were lowest at 900 mt as confirmed by the lowest total acidity. Citric acid were not detected.

Neochlorogenic and p-coumaroylquinic acids were the main hydroxycinnamic acid derivatives, with the highest concentrations in fruits picked up at 500 meters. The LC-MS analysis evidenced

cyanidin-3-rutinoside as main anthocyanins (84.79%), while total anthocyanins content was higher in cherry fruits at 900 mt.

The results showed that sweet cherries "Mastrantonio" are influenced by altitude in their nutraceutical characteristics. In fact total anthocyanins content was higher at 900 mt (87.61 mg/100g), while phenols and antioxidant activity were higher at 500 mt (91.61 mg/100mg GAE; 3123.67 μmol/100g) respectively.

Introduction

Sweet cherry is an important fruit with high commercial importance in Sicily, where in the last thirty years there has been a strong development thanks to the introduction of new orchards and new rootstock. Previous studies indicated as different rootstocks could induce different effects regarding the tree vigor and physiology as flavonol and phenolic compound concentrations (Usenik et al. 2002, Gonçalves et al. 2006, Jakobek et al. 2009).

On the north-east side of Etna mountain are produced four different typical cultivars "Mastrantonio", "Napoleona", "Raffiuna" and "Maiolina" well known and appreciated by consumers for their quality characteristics of sweetness, skin colour and firmness.

Cherries are considered to be a major source of phenolic compounds, which are also responsible for their colour and taste and presumably also their antioxidant properties (Gonçalves et al. 2006).

A special interest to increase the daily intake of fruit and vegetables has been associated with reduced incidence of degenerative diseases due to anthocyanins and polyphenolics antioxidant properties (Serrano et al. 2009).

Anthocyanins constitute a large family of polyphenols in plants and are responsible for many of the fruit and floral colours observed in nature.

The major phenolic antioxidants in sweet cherries are anthocyanins but sweet cherries also have significant amounts of phenolic acids and flavonols (Jakobek et al. 2009). Among hydroxycinnamates, sweet cherries have neochlorogenic and p-coumaroylquinic acid as the predominant compounds (Kim et al. 2005).

Nowadays HPLC coupled with MS had become a very efficient tool for the characterization and elucidation of unknown and partially unknown anthocyanins pigments in fruits and vegetable (Chandra et al., 2001; Dugo et al., 2001).

The antioxidant activity explained by phenolic compounds and anthocyanins has been surveyed using the oxygen radical absorbance capacity (ORAC) assay.

Quality characteristics, nutritive and bioactive compounds of sweet cherry at the time of harvest differ among cultivars, as reported by previous studies (Serrano et al. 2009) and there is no additional information about the relation between these characteristics with the altitudes of growing.

The aim of the present work was to identify the main quality characteristics of "Mastrantonio" sweet cherry harvested at ripening time and to quantify chemical attributes (the content of sugars, organic acids, total and individual anthocyanins, total phenolic content and antioxidant activity) to find correlations between them and the three different altitudes tested.

Materials and Methods

The study was carried out on sweet cherries (Prunus avium L.) cultivar "Mastrantonio", collected from orchard on the north-east side of Etna mountain at three different altitudes 500, 600 and 900 meters. Sweet cherries fruits were harvested at the ripe stages in June (500 m., 600 m.) and July (900 m.). For each altitude, 3 replicates of 120 fruits each were selected. The following maturity and quality indices:

weight, texture (or flesh firmness), equatorial and longitudinal diameter and skin colour, according to CIE Lab scale using a Minolta CR200 colorimeter (Minolta, Milano, Italy) were assessed on the fresh fruits.

The remaining samples were packed in plastic bags, frozen and kept at -80°C until extraction.

Chemicals and Reagents

The organic acids: malic acid, citric acid, fumaric acid and shikimic acid; the phenolic compounds gallic acid, chlorogenic acid, neochlorogenic acid, p-coumaroylquinic acid were purchased from Sigma Chemical Co.

Anthocyanins standard (cyanidin-3-O-glucoside chloride, cyanidin-3-O-solphuroside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside) were purchased from Extrasynthese (Geney, France).

Ethyl acetate and methanol of analytical grade were used for extraction, and methanol and acetonitrile of HPLC-MS grade were used for analysis.

Chemical Analysis

Total soluble solid (TSS), total acidity (TA) (as anhydrous citric acid) and pH were determined according to standard method (MAF, 1989; Kimball, 1999). An automatic pH titration system (Mettler Toledo) was used for pH determination.

Fruit samples were analyzed for the content of individual sugars (glucose, fructose and sucrose) and organic acids (malic, citric, fumaric and shikimic) as reported by Usenik et al. (2008) and Sturm et al. (2003). The fruits were stoned and homogenized with a manual blender. Mashed fruit were centrifuged at 15000rpm for 20 min at 4°C. The supernatant was filtered through Miracloth filter paper then

passed through a C18 Sep-Pak cartridge (Waters) previously activated with CH_3OH and H_2O, and filtered through a 0.45 μm filter. Sugars and organic acids were determined by using the high-performance liquid chromatography device: Waters mod. 600-E liquid chromatograph (Milano, Italy), equipped with a PDA Waters 996 detector and managed by Millennium 3.2 Waters software. Separation of sugars was carried out using a Phenomenex Luna NH_2 column (250 x 4.60 mm) in isocratic conditions acetonitrile/water (80:20, v/v) with 1.8 ml/min as flow rate. Analysis of organic acids was carried out using a Phenomenex Synergi 4u Polar-RP column (150 x 4.60 mm ID) thermostated at 35°C in isocratic conditions with 0.7 ml/min as flow rate with a 4 mmol sulphuric acid in solution of water:acetonitrile (90:10) used as eluent. Organic acids were identified and quantified by using a UV detector with wavelength set at 210 nm.

Hydroxycinnamic acids (neochlorogenic and p-coumaroylquinic acid) were extracted from juice by solid-phase extraction (SPE) after alkaline hydrolysis of hydroxycinnamic esters and analyzed by HPLC (Rapisarda et al. 2001).

A 10-mL sample of centrifuged juice was added to 10 mL of 2 N sodium hydroxide and stored at room temperature in the dark. Complete hydrolysis of bound hydroxycinnamic acids occurred in 4 h. The solution was then acidified with 2 N chloridric acid (HCl) to pH 2.5 and passed through a C18 SPE cartridge (Waters, Milford, MA). Hydroxycinnamic acids were eluted with 0.1% HCl in methanol. The alcoholic solution was 0.45-μm filtered, and 20 μL of the solution was analyzed by HPLC equipped as above. The eluents used were water:acetic acid (98:2) (solvent A) and methanol (solvent B) with a gradient transition from 95% to 70% of solvent A over 35 min. The flow rate was 1 mL/min, and detection was performed at 300 nm.

The extraction of phenolic compounds were performed as reported by Tomas-Barberan et al. (2001). Briefly 5g of frozen fruits were

homogenized with 10 ml of extraction solution (water/methanol 2:8 containing 2 mM NaF); homogenates were kept in ice until centrifuged (12000 rpm, 15 min, 4°C). The extracts obtained were analyzed for their total phenolic content according to the Folin-Ciocalteu (FC) colorimetric method (Singleton, 1999). One ml aliquot of the diluted sample (1:20 with H_2O) was mixed with 5 ml FC commercial reagent (previously diluted with H_2O 1:10 v/v) and 4 ml of a 7.5% Na_2CO_3 solution. Mixture was stirred for 2 h at room temperature and away from strong light. The absorbance of the resulting blue color was measured spectrophotometrically at 740 nm and the concentration of total phenols was expressed as gallic acid equivalents (mg/100g).

Total anthocyanins were determined spectrophotometrically (Varian UV/Vis spectrophotometer mod. Cary 100 Scan) by the pH differential method (Rapisarda et al., 2000). The content of total anthocyanins was expressed in mg cyanidin-3-glucoside equivalents (CGE)/100 g of fresh cherry.

For the individual anthocyanins identification the sweet cherry juice was centrifuged at 15000rpm for 20 min at 4°C, then supernatant was loaded onto preconditioned C-18 Sep-Pak cartridges, eluted with 1% formic acid methanol then rotary evaporated. The residue was dissolved in 2 ml of distilled water and extracted using ethyl acetate. The ethyl acetate extract was evaporated and the residue was dissolved in 7% aqueous formic acid (Vasco et al., 2009).

Individual anthocyanins identification was performed by HPLC-DAD-MS/MS. Separation of anthocyanins was conducted on a Merck Chromlith Performance RP-18e column (100-3 mm) using a Ultra Fast HPLC system coupled to a photodiode array (PDA) detector and a Finnigan LXQ ion trap equipped with an electrospray ionization (ESI) interface, in a series configuration (Thermo Electron Corporation, San Jose, CA). A binary gradient composed of 2%

aqueous formic acid (solvent A) and acetonitrile/water/formic acid (80:18:2, v/v/v; solvent B) was used (Mertz et al., 2007). The solvent system was: A, water:formic acid (85:15); B, water:formic acid:acetonitrile (35:15:50). Anthocyanins were analyzed using the following gradient: from 5 to 25% B in 60 min and from 25 to 5% in 10 min at a flow rate of 0.3 mL/min, followed by re-equilibrating the column to initial conditions. The injection volume was 20 μL. The isolation width was 1.4 (m/z) and the normalized collision energy 19.0 (V); the MS conditions were: source voltage 5.50 KV, capillary temperature 275 °C, capillary voltage 19 V.

The UV-Vis absorption chromatogram was detected at 520 nm and the column temperature was maintained at 25 °C. MS full-scan acquisition (m/z 150-1205) was first performed in a positive mode. Then, chosen peaks were isolated in the ion trap and fragmented by an MS-MS full scan acquisition.

The peroxyl radical scavenging efficacy of the same extracts obtained for total phenols analysis was determined using the ORAC assay, as described by Ou, Hampsch-Woodill, and Prior (2001), with some modifications. Briefly, the measurements were carried out on a Victor III 96-well plate reader (Perkin-Elmer, Massachusetts, USA) with a fluorescence filter (excitation 485 nm, emission 535 nm), the Wallac 1420 software is used to control the system. Fluorescein (116 nM) was the target molecule for free radical attack from AAPH (153 mM) that was used as the peroxyl radical generator.

Statistical analysis

Data were analyzed by variance analysis (ANOVA) and mean separation was determined by Tukey's test with STATSOFT 6.0.

Results

Physicochemical results are reported in Table 1. Samples had a middleweight and medium-high size. It's well known that the most important parameters determining sweet cherry acceptability by consumers are bright red color and flavor, which is mainly due to the ratio between TSS and TA (Crisosto, 2003). Small differences are observed between the three different altitude, and when these are present and statistically relevant they concern fruits harvested at 600 and 900 meters of altitude. Sweet cherry picked up at 900 meters shown the highest TSS value and color parameter. Total solid soluble content was lowest in sweet cherry grown at 600 meters, as expected, considering the lowest concentrations of glucose, fructose and sucrose detected in this sample.

The amount of glucose (5.82 and 4.32) and fructose (6.09 and 4.59) were similar in all samples, with a statistic difference between fruits picked up at 500 and 600 meters respectively. Sucrose content was similar in fruits at 500 and 900 meters.

Malic acid is the most representative organic acids in sweet cherry, in agreement with previous paper (Serrano et al. 2005; Usenik et al. 2008), with different percentages of shikimic and fumaric acid that shown variations among cultivars (Usenik et al. 2008).

Amount of malic and fumaric acid were lower in fruits picked up at the highest altitude; this attitude was reflected also in the total acidity values.

The differences in the concentration of malic acid at the three different altitude are not significative for "Mastroantonio" whereas citric acid was not detectable (only trace amounts of citric acid were detected).

The obtained HPLC profiles of hydroxycinnamic acids were identified and the area of chromatographily monitored hydroxycinnamic acids was converted to chlorogenic acid equivalents with external calibration as suggested by Mozetic et al. (2002, 2006). The sum of

both neochlorogenic and p-coumaroylquinic acid was presented as total hydroxycinnamic acids.

The content of two hydroxycinnamic acids varied among different cultivars and in “Mastroantonio” picked up at 500 meters we had the highest values for both, but the p-coumaroylquinic acid was the most abundant with a significative statistical difference from samples of the other two altitude. As expected the total hydroxycinnamic acids content showed the same trend discussed above.

Total phenols data are in line with those reported in previous studies (Usenik et al., 2008; Ferretti et al., 2010); these data as results coming from antioxidant activity assay were highest in cherries at 500 and 600 meters, while total anthocyanins were highest at the highest altitude of production.

The ORAC assay confirmed that cherry had a high antioxidant capacity; can be observed that ORAC values decrease with an increasing of the altitude, in fact cherry harvested at 500 mt showed the higher (3123.67 μmol Trolox/100 g) values were in the range of results obtained in previous paper (Kevers et al., 2007).

To better characterize our cultivar, anthocyanins were identified through LC/MS (Mozetic et al., 2006), the HPLC chromatogram is shown in Fig.1, while retention time molecule ions and main fragments of the anthocyanins pigments are reported in Table 2. The five anthocyanins analyzed representing more than 90% of the total peak area, were identified with the assistance of UV-Vis spectra, MS^n and literature data (Bonerz et al., 2007).

The most abundant was cyanidin-3-rutinoside as reported in previous papers (Kim et al., 2005; Fazzari et al., 2008), followed by cyanidin-3-glucoside and peonidin-3-rutinoside. Amount of cyanidin-3-sophoroside and pelargonidin-3-rutinoside were detected in tracks.

Table 1. Physicochemical evaluation

	Altitude		
	500	**600**	**900**
Weight (g)	9.32[a]	7.68[b]	8.46[a]
Texture (kg)	0.63[b]	0.74[b]	0.97[a]
Equatorial diameter (mm)	25.6[a]	24.17[b]	24.31[b]
Longitudinal diameter (mm)	25.44[a]	24.17[b]	25.24[a]
L*	24.95[ab]	24.47[b]	25.52[a]
a*	6.3[ab]	5.29[b]	7.83[a]
b*	1.49[ab]	1.21[b]	2.08[a]
Total Soluble Solid (°Brix)	19.72[ab]	18.73[b]	20.65[a]
Total Acidity (% citric acid)	0.38[a]	0.35[a]	0.29[b]
pH	4.72[a]	4.42[b]	4.78[a]
Sugar (g/100ml)			
Glucose	5.82[a]	4.32[b]	4.83[ab]
Fructose	6.09[a]	4.59[b]	5.08[ab]
Sorbitol	3.75[a]	1.93[b]	3.06[a]
Organic Acids (mg/100g)			
Malic acid	445.2	445.38	401.73
Shikimic acid	3.44[b]	4.47[a]	4.24[ab]
Fumaric acid	0.57[a]	0.41[a]	0.34[b]
Citric acid	-	-	-
Hydroxycinnamic acids (mg chlorogenic acid equivalent/100g)			
Neochlorogenic acid	11.93[a]	8.5[a]	4.66[b]
p-coumaroylquinic acid	14.59[a]	11.77[b]	12.22[b]
Total hydroxycinnamic acids	28.43[a]	22.27[a]	18.4[b]
Total phenols (GAE mg/100g)	91.61[a]	83.93[a]	77.68[b]
Total anthocyanins (CGE mg/100g)	64.47[b]	72.43[a]	87.61[a]
ORAC (µmol Trolox/100g)	3123.67[a]	2701[a]	2283.3[b]

Different letters indicate significant differences at $p \leq 0.05$; no letters no significant

Table 2. Anthocyanins identification through LC/MS

Peak	RT (min)	M^+ (m/z)	MS^n	Anthocyanins	%
1	23.96	611	449/287	cyanidin-3-sophoroside	0.82
2	25.07	449	287	cyanidin-3-glucoside	7.56
3	26.1	595	449/287	cyanidin-3-rutinoside	84.79
4	29.15	579	433/271	pelargonidin-3-rutinoside	0.73
5	30.85	609	463/301	peonidin-3-rutinoside	3.83

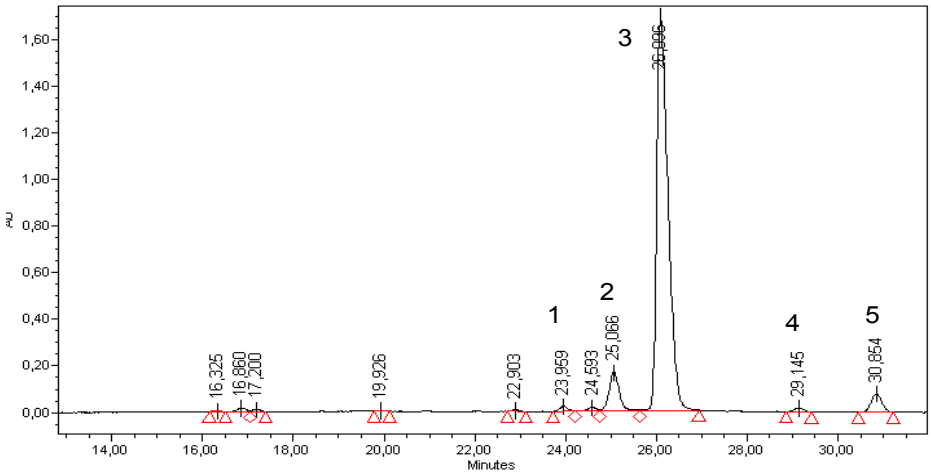

Figure 1. Anthocyanins HPLC chromatogram

References

Bonerz D., Würth K., Dietrich H., Will F., 2007. Analytical characterization and the impact of ageing on anthocyanin composition and degradation in juices from five sour cherry cultivars. Eur. Food Res. Technol. 224, 355-364.

Chandra, A., Rana, J., Li, Y. 2001. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. J. Agric. Food Chem. 49, 3515-3521.

Crisosto, C.H., Crisosto G.M., Metheney P. 2003. Consumer acceptance of “Brooks” and “Bing” cherries is mainly dependent on fruit SSC and visual skin color. Postharvest Biol. Technol. 28, 159-167.

Dugo, P., Mondello, L., Errante, G., Zappia, G., Dugo, G. 2001. Identification of anthocyanins in berries by narrow-bore high performance liquid chromatography with electrospray ionization detection. J. Agric. Food Chem. 49, 3987-3992.

Fazzari M., Lana Fukumoto L., Mazza G., Livrea M.A., Tesoriere L., Di Marco L., 2008. In vitro bioavailability of phenolic compounds from five cultivars of frozen sweet cherries (*Prunus avium* L.) J. Agric. Food Chem., 56 (10), 3561–3568

Ferretti G., Bacchetti T., Belleggia A., Neri D., 2010. Cherry antioxidants: from farm to table. Molecules 15, 6993-7005.

Gonçalves B., Moutinho-Pereira J., Santos A., Silva A.P., Bacelar E., Correia C., Rosa E. 2006. Scion-rootstock interactions affects the physiology and fruit quality of sweet cherry. Tree Physiol. 26, 93-104.

Jakobek L., Seruga M., Voca S., Sindrak Z., Dobricevic N. 2009. Flavonol and phenolic acid composition of sweet cherries (cv. Lapins) produced on six different vegetative rootstocks. Scientia Horticulturae 123, 23-28.

Kevers C., Falkowski M., Tabart J., Defraigne J.O., Dommes J., Pincemail J., 2007. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J. Agric. Food Chem., 55 (21), 8596-8603.

Kim D.O., Heo H.J., Kim Y.J., Yang H.S., Lee C.Y. 2005. Sweet and sour cherry phenolics and their protective effects on neuronal cells. J. Agric. Food Chem. 53, 9921-9927.

Kimball, D., 1999. Citrus Processing. Quality Control and Technology. AVI Books, New York.

MAF. Metodi ufficiali d'analisi per le conserve vegetali (Official Analysis Methods for Plant Preserves). Parte generale, D.M., Feb. 3, 1989.

Mertz C., Cheynier V., Gunata Z., Brat P., 2007. Analysis of phenolic compounds in two blackberry species (Rubus glaucus and Rubus adenotrichus) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. J. Agric. Food Chem. 55, 8616-8624.

Mozetic B. et al. 2002. Anthocyanins and hydroxycinnamic acids in sweet cherries, Food Technol. Biotechnol., 40 (3), 207-212

Mozetic B., Simcic M., Trebse P., 2006. Anthocyanins and hydroxycinnamic acids of Lambert Compact cherries (Prunus avium L.) after cold storage and 1-methylcyclopropene treatment. Food Chem. 97, 302-309.

Ou B., Hampsch-Woodill M., Prior, R. L. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49, 4619-4926.

Rapisarda, P., Fanella, F., Maccarone E. 2000. Reliability of analytical method for determining anthocyanins in blood orange. J. Agric. Food Chem. 48, 2249-2252.

Serrano M., Diaz-Mula H.M., Zapata P.J., Castillo S., Guillen F., Martinez-Romero D., Valverde J.M., Valero D. 2009. Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. J. Agric. Food Chem. 57, 3240-3246.

Serrano M., Guillen F., Martinez-Romero D., Castillo S., Valero D. 2005. Chemical Constituents and Antioxidant Activity of Sweet Cherry at Different Ripening Stages. J. Agric. Food Chem., 53, 2741–2745.

Singleton V.L., Orthofer R., Lamuela–Raventos R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteau reagent, Methods in Enzimolgy, 299, 152-178.

Sturm K., Koron D., Stampar F. 2003. The composition of fruit of different strawberry varieties depending on maturity stage. Food Chemistry 417-422.

Tomas-Barberan, F.A.; Gil, M.I.; Cremin, P.; Waterhouse, A.L.; Hess-Pierce, B.; Kader, A.A. 2001 HPLC-DAD–ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J. Agric. Food Chem. 49, 4748-4760.

Usenik V., Fabeic J., Stampar F., 2008. Sugar, organic acids, phenolic composition and antioxidant activity of sweet cherry. Food Chemistry 107, 185-192.

Usenik V., Stampar F., 2002. Influence of scion/rootstock interaction on seasonal changes of phenols. Phyton-Ann. REI Bot. 42, 279-289.

Vasco C., Riihinen K., Ruales J., Kamal-Eldin A. 2009. Phenolic compounds in Rosaceae fruits from Ecuador. J. Agric. Food Chem. 57, 1204-1212.

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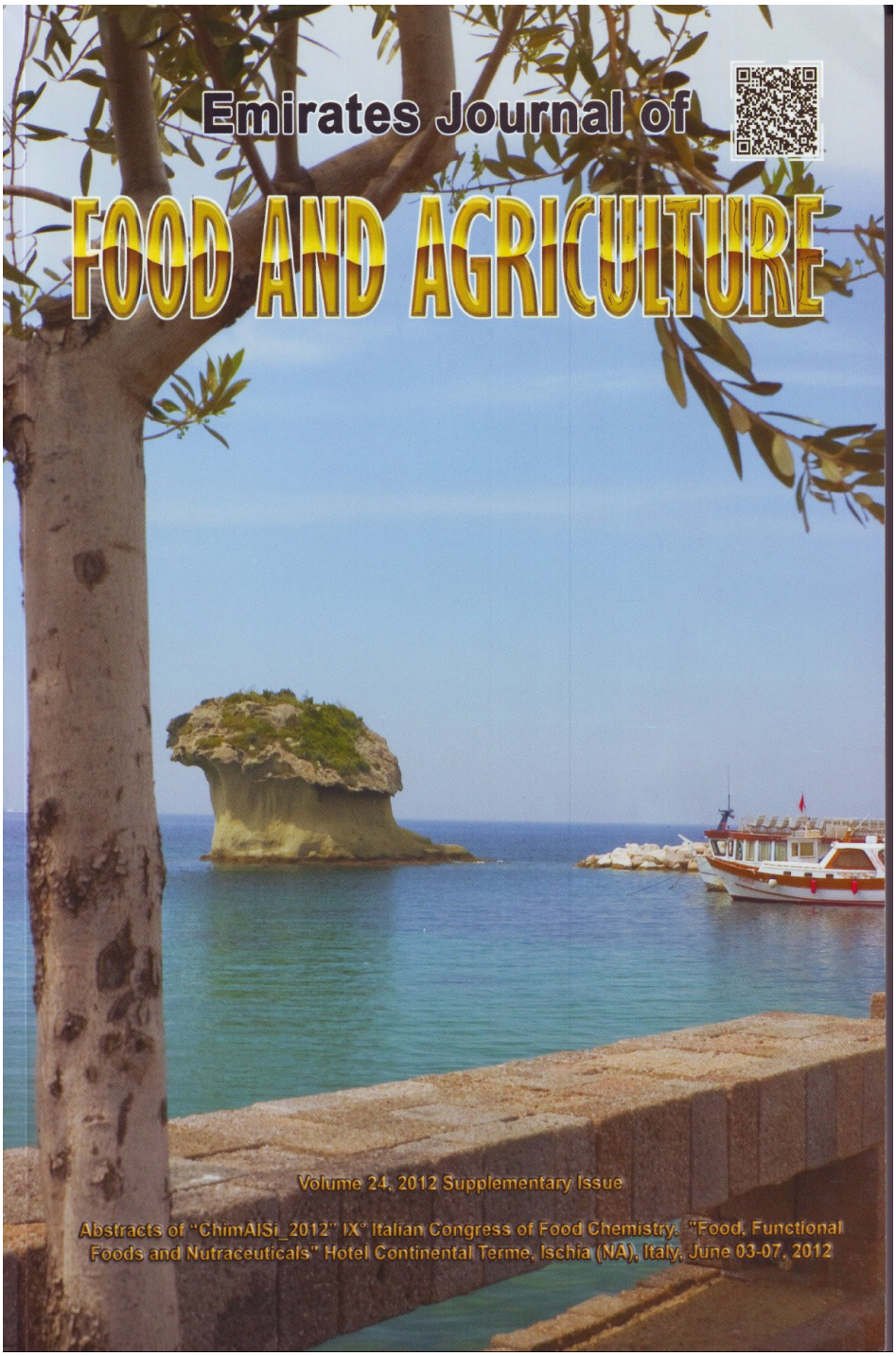
Emirates Journal of
FOOD AND AGRICULTURE
Volume 24, 2012 Supplementary Issue
Abstracts of "ChimAlSi_2012" IX° Italian Congress of Food Chemistry. "Food, Functional Foods and Nutraceuticals" Hotel Continental Terme, Ischia (NA), Italy, June 03-07, 2012

Emirates Journal of Food and Agriculture
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Hotel Continental Terme, Ischia (NA), Italy, June 03-07, 2012

P-176

High throughput quantitative analysis of multi mycotoxin in beer-based drinks using UHPLC-MS/MS

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Abstract
Mycotoxins often exist as contaminants in grains. Meanwhile, in response to consumer needs for food safety, food and beverage manufactures have to strictly manage the risks of such contaminants. Therefore, it is essential for the management of high-quality to rapidly determine the concentrations of mycotoxins in foods or beverages. UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. The high throughput method for the quantification of 14 mycotoxins in beer-based drinks had been developed. While high sensitivity analysis is performed, carry over becomes a problem due to the adverse effects on LC-MS/MS high- sensitivity analysis. For eliminating carry over, rinse condition of autosampler was also examined. Standards of 14 mycotoxins (patulin, nivalenol, deoxynivalenol, 4 aflatoxins, T-2 toxin, HT-2 toxin, zearalenone, 3 fumonisins and ochratoxin A) were optimized on each compound-dependent parameter and MRM transition (Q1/Q3) and then they were analyzed on LC-MS/MS condition as follows. Nexera HPLC system was connected to LCMS-8030 triple quadrupole mass spectrometer. Chromatographic separation was carried out using ODS column, TriartC18 (2.0 mmI.D., 100 mm, 1.9um) maintained at 40C. Mobile phase consisted of: solvent A, ammonium acetate-water; and solvent B, acetic acid-methanol. And sample was applied to MS/MS with electro ion spray source, then analyzed with positive and negative MRM mode. As for Nexera autosampler, multiple rinse modes with two rinse solvents were examined to eliminate sample carry over.

P-177

Fruit quality and human health-bioactive compounds of sweet cherry (*Prunus avium* l.) cultivars grown in Italy

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Abstract
This research was undertaken to evaluate the fruit quality parameters (fruit weight, total soluble solids, titratable acidity, firmness and colour), phenolic compounds and antioxidant capacities of twenty four sweet cherry (*Prunus avium* L.) cultivars grown on the mountainsides of the Etna volcano (Sicily, Italy). High-performance liquid chromatographic methods were used to identify and quantify sugars (sucrose, glucose, fructose and sorbitol) and organic acids (malic, citric, shikimic, and fumaric acid). A total of eight phenolic compounds were identified and quantified in sweet cherry cultivars, including three hydroxycinnamic acid derivatives (neochlorogenic acid, *p*-coumaroylquinic acid and chlorogenic acid) and five anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside). The relative amounts of the phenolic compounds varied widely across the cherry cultivars examined in this study. Total anthocyanin contents ranged from 4.58 to 94.20 mg of cyanidin-3-glucoside equivalents/100 g of FW, while total phenolic contents ranged from 81.62 to 166.03 of gallic acid equivalents/100 g of FW. The ORAC assay indicated that fruit of all genotypes possessed considerable antioxidant activity. The high level of phenolic compounds and antioxidant capacity of sweet cherry fruits studied implied that they might be sources of human health-bioactive compounds.

PUBL.5


Dipartimento di Scienze degli Alimenti e dell'Ambiente
"Prof. G. Stagno d'Alcontres"
VIII
Congresso Nazionale di Chimica degli Alimenti
Qualità e Tipicità degli Alimenti Mediterranei: Alimentazione e Salute
20 - 24 Settembre 2010
Marsala
Società Chimica Italiana
Riassunti
a cura di
Giuseppa Di Bella, Vincenzo Lo Turco,
Nicola Cicero e Angela Giorgia Potortì

QUALITA' E PROPRIETA' FUNZIONALI DI FRUTTI DI CILIEGIO CV "MASTRANTONIO"

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Da oltre trent'anni la cerasicoltura italiana vive in continua evoluzione, con un forte sviluppo di nuovi impianti contraddistinti da una minore dimensione dei fruttiferi grazie a specifiche forme di allevamento e all' uso di nuovi portinnesti.

Nella provincia di Catania la coltivazione è ubicata prevalentemente lungo il versante nord-orientale dell'Etna dai 200 m s.l.m. fino ai 1.300 m s.l.m.. In quest'area sono presenti diversi ecotipi locali quali la "Mastrantonio", la "Napoleona", la "Raffiuna" e la "Maiolina", tutti facenti parte della DOP "Ciliegia dell'Etna" [1]

Tra i vari ecotipi particolare interesse riveste la cv "Mastrantonio", che, grazie alle specifiche caratteristiche organolettiche dei frutti, riscuote un buon gradimento da parte dei consumatori. E' una varietà molto produttiva la cui epoca di maturazione va da metà giugno a metà luglio a seconda dell'altitudine a cui viene coltivata. Ad oggi non risultano in letteratura lavori scientifici sulle proprietà funzionali dei frutti di questa varietà.

La presente ricerca ha avuto come obiettivo la caratterizzazione qualitativa di frutti raccolti a maturazione a diverse altitudini (500 m., 600 m., 900 m.), volta a evidenziare le proprietà nutrizionali e salutistiche utili per la valorizzazione e la promozione del prodotto. Sono stati valutati i parametri classici della qualità dei frutti (peso medio, colore, solidi solubili totali, acidità totale e pH) ed altri indici, importanti per le loro proprietà biologiche (zuccheri, acidi organici, vitamina C, antocianine, polifenoli totali, e l'attività antiossidante totale mediante saggio ORAC).

I frutti presentano peso medio e dimensioni medio-alte. Il colore si differenzia in relazione all'altitudine con valori di L*, a*, b*, più elevati nei frutti raccolti a 900 m. I livelli di solidi solubili più bassi sono stati osservati nei frutti coltivati a 600 metri, determinati certamente dalle più basse concentrazioni di glucosio, fruttosio e, in particolare, di sorbitolo in questi campioni. I contenuti di acido malico e fumarico sono risultati più bassi nei frutti raccolti a maggiore altitudine e questo si è riflesso nei valori di acidità totale.

L’analisi HPLC-MS delle antocianine ha rivelato la prevalenza della cianidina-3-glucoside e di minori quantità di cianidina-3-soforoside, cianidina-3-rutinoside e peonidina-3-rutinoside.

Il contenuto di polifenoli totali è risultato decisamente elevato in tutti i campioni analizzati, con livelli più alti nei frutti raccolti a più bassa quota (167,03 mg/100 g). Questo parametro ha influenzato i valori dell’attività antiossidante che sono risultati superiori a 2500 µmoli/100 g. In conclusione, i risultati ottenuti mettono in evidenza le proprietà funzionali dei frutti di ciliegio della varietà “Mastrantonio”.

Bibliografia:

[1] Disciplinare di Produzione “CILIEGIA DELL’ETNA” DOP. Regolamento CE 510/06

PUBL.6

II ISHS International Conference on Quality Management of Fresh Cut Produce: *Convenience Food for a Tasteful Life*

17-21 July 2011, Turin - Italy

Freshcut2011

Shelf-life of Minimally Processed Table Grapes Packed in Snack-size Containers

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Keywords: Packaging, quality attributes, phenolics, ORAC, antioxidant activity.

Abstract

Minimally processed table grapes have traditionally being used as component of fruit salads; only recently the possibility to produce single-portion packed in small containers has been taken into account. The aim of the research was to assess the shelf-life of small size table grape clusters of some of the most diffused varieties in Italy such as 'Vittoria', 'Sugraone', 'Italia', 'Crimson Seedless', 'Red Globe' and 'Black Pearl' packed in polyethylene containers. After packaging, containers were stored at 4±1 °C and physicochemical and microbiological parameters were monitored after 0, 7 and 15 days of storage. The berry firmness remained satisfactory until the 15th day of storage, especially in seedless cultivars such as 'Sugraone' and 'Crimson seedless'. Variations of weight loss and color were almost absent in all cultivars. A significant increase of CO_2 and decrease of O_2 inside the packages were observed during the second week of storage. As known, CO_2 increase plays an important role in preventing the development of mesophilic bacteria. During the cold storage period, most of the quality parameters *remained* stable in all the cultivars. Ascorbic acid and total phenolic compounds content decreased, showing significant differences after 7 days of storage. Antioxidant activity showed a decline as seen for ascorbic acid and total phenolic compounds content. The results indicated the possibility of packaging small size clusters of table grape maintaining good quality parameters for a rather long period of storage without determining excessive reduction of the antioxidant activity of the product.

INTRODUCTION

Table grape industry in Italy is currently involved in a process of economic contraction due to the change of the world scenary: there is a significant regression of the European productions in comparison to those coming from Asia and other emergent countries. This dynamism has strongly conditioned the Italian productions that find

several difficulties in the international markets and are less remunerate in comparison to the foreign ones. Due to this dynamism it is really important to find innovations that can support the competitiveness of the productions within the Italian and foreign markets. An important impulse to the commercial diversification is driven from the consumers, that require a high-level qualitative product regarding nutritional, sensorial and hygienic sanitary profile; moreover today, due to life-style and alimentary habits, there is an increasing request of products ready for the consumption and with a sustainable cost.

The possibility to introduce innovation such as fresh-cut table grapes represents an opportunity for table grape growers and consumers. This innovation could increase the product value, but sets different problems concerning the grape aspect under physical, chemical and biological profile. Table grape is a nonclimateric fruit that shows severe problems during postharvest handling, storage, and marketing. As with other fruits, weight loss, color changes and accelerated softening affect product quality. Additionally, the postharvest quality deterioration in table grapes is also attributed to rachis browning and high incidence of berry decay (Carvajal-Millàn et al., 2001; Crisosto et al., 2002). Some of the quality traits of table grape, such as berry size and skin thickness, can represent limiting factors when preparing minimally processed products. Further difficulties found in the preparation of fresh-cut table grapes easily are due to the delicacy of the clusters subjects to mechanical damages during harvest, washing and packaging and also from the microbial attacks. Greater care in handling and packaging grapes can ensure a longer shelf life and thus increase consumer confidence in the product (Piva et al., 2006). Microbial attacks can easily be found in badly dried and damaged grapes. Drying represents a critical point due to the fact that neither the centrifugation nor the forced air can be used (Lovino et al. 2003). Besides these aspects packaging plays an important role since the containers must be transparent, strong, manageable and sealed with a suitable film.

The aim of this research, carried out in the years 2009/2010, was to assess the shelf-life of small size table grape clusters of the most diffused varieties in Italy packed in polyethylene containers and sealed using polyamide-polyethylene film. In addition, quality attributes, total phenolic compounds, vitamin C and antioxidant activity of different grape varieties were evaluated during cold storage.

MATERIALS AND METHODS

Table grape clusters ,in optimum ripeness stage, (20 Kg for each cultivar ‘Vittoria’, ‘Sugraone’, ‘Italia’, ‘Crimson seedless’, ‘Red Globe’ and ‘Black Pearl’) were harvested from 10 years old vineyards, located in the Sicilian protected Geographical

Identification (PGI) area of Mazzarone (CT). Once in the laboratory, the main morphological values were measured and then clusters were selected to obtained homogeneous batches based on color, size, absence of injuries, and healthy greenish rachises. Clusters were washed in a chloridric (5-20 ppm) and citric acid (80 ppm) solution and dried in a laminar flow. Morphological parameters (berry weight, diameter, volume) were recorded. The clusters were cut to obtain small size table grape in the range of 30-50 g and packaged in polyethylene containers (10x10cm) sealed using polyamide-polyethylene film (Niederwieser, Bolzano). Fifteen packages for each grape variety were stored at 4 °C with 90% RH and sampled at 0, 7 and 15 d of cold storage. For each sampling date, 5 trays for each grape variety were used for the physicochemical analysis. Gas composition, O_2 and CO_2 percentages, inside the containers during storage period, was determined by Dansensor PBI (Ringsted, Denmark).

Berry firmness was determined using a TX-XT2i Texture analyzer (Stable Microsystems, Godalming, U.K.). Color was evaluated using a Minolta colorimeter CR410 (Minolta Camera Co., Osaka, Japan). Color was expressed as L* a* b* for all samples and also as hue angle for red and black cultivars. Weight of each tray was recorded on the packaging day and after the different sampling dates. Weight losses were expressed as percentage of original weight. Total soluble solid content (TSS) was determined in the juice obtained from 10 berries for each tray with a digital refractometer. Titratable acidity (TA) was determined in juice by potentiometric titration with 0.1 N NaOH up to pH 8.1, and expressed as g of tartaric acid equivalent per 100 mL^{-1}.

Fructose and glucose were analyzed using a Waters 600E HPLC system. Centrifuged grape juice (5 mL) was passed through a Sepak-C18 cartridge and 1 mL of cleaned juice was diluted to 50 mL with double-distilled water. Sugars were detected using a Waters 410 Differential Refractometer detector with reference cell maintained at 35°C. A Luna 5µ NH_2 column (250 x 4.6mm) was used. The column was maintained at 30°C with a Waters thermostated column compartment. Sample were eluted with CH_3CN-H_2O (80-20) solution and the flow rate was 1.8 mL/ min.

Vitamin C was determined by an AOAC (1990) procedure with 2,6dichloroindophenol titration method. The total phenolic concentration of fruit, expressed as mg of gallic acid equivalent (GAE)/100g of berry, was measured using Folin- Ciocalteu reagent assay (Singleton et al., 1999). Grape berries (50 g) were ground in liquid N_2 and recovered into 100 mL flask with H_2O-MeOH (2:8) containing 2 mmol/L NaF to inactivate polyphenol oxidases. After centrifugation the supernatant was analyzed for total phenolic compounds. The same extract was used for total antioxidant activity (TAA) determination, following the ORAC assay, as described by Ou,

et al. (2001), with some modifications. Briefly, the measurements were carried out on a Wallac 1420 Victor III 96-well plate reader with a fluorescence filter (excitation 485 nm, emission 535 nm). Fluorescein (116 nM) was the target molecule for free radical attack from AAPH (153 mM) used as the peroxyl radical generator. The reaction was conducted at 37 °C and pH 7.0. All solutions were freshly prepared prior to analysis. All samples were diluted with phosphate buffer (1:25–100, v/v) prior to analysis and results were reported as micromoles of Trolox equivalents per 100 g of fresh weight.

Analysis of variance (ANOVA), performed by STATSOFT 6.0, was used to test the significance of each variable ($p< 0.01$) during storage and the means separation was executed by Tukey test. Multivariate analysis (Linear Discriminant Analysis) was carried out using SPSS-95 statistical package.

RESULTS AND DISCUSSION

During storage a significant O_2 decrease was observed after 7 days in the trays headspace for all the varieties. The O_2 decrease was balanced by a strong increase of CO_2 during 7 days of cold storage. Minimally processing has led to an increase of respiratory activity in grapes, which created a gas atmosphere rich in CO_2 and poor in O_2 inside the trays. 'Vittoria' and 'Red Globe' varieties showed a lower respiratory activity (data not shown).

The percentage of weight loss during storage was very low for all varieties with a range of 0.5 – 1.0 %. The quality parameters determined in the different grape varieties are shown in Table 1. The berry firmness remained satisfactory until the 15th day of storage in all grapes studied. The values at T0 were higher in the seedless varieties 'Sugraone' and 'Crimson' and in 'Black Pearl'grape. 'Red Globe' and 'Black Pearl' showed a significant decay between T0 and T15, whereas firmness of 'Italia' variety declined already after 7 days of storage. 'Sugraone', 'Vittoria' and 'Crimson', didn't show any variation during storage. Color of skin remained unchanged during storage, also in the white varieties (data not shown).

TSS content is commonly used to evaluate the quality of table grapes and also to determine the harvest maturity. The TSS values of the 6 varieties ranged from 14.54% ('Vittoria') to 20.65% ('Black Pearl'), high values were also recorded in 'Italia' (20.18%) and 'Red Globe' (20.17%) cultivars. During storage a change of TSS between T0 and T7 was observed only in 'Red Globe' variety. The major sugars found were fructose and glucose, with traces of sucrose (data not shown). Fructose and glucose concentrations of 'Crimson', 'Red Globe' and 'Black Pearl' declined during first 7 days of storage, then a light increase of these parameters was noted in 'Crimson' and 'Vittoria'

(only fructose) varieties. This may be due to the evaporation phenomena resulting from long-term storage.
Titratable acidity (TA) at harvest ranged from 0.42 g/100mL (‘Red Globe’) to 0.67 g/100mL (‘Black Pearl’). A decrease of this parameter throughout storage was observed in ‘Vittoria’ and ‘Crimson’ varieties. Jayasena and Cameron (2008) found that consumer liking of grapes substantially changed with a change in acidity and consumer acceptance of table grape increased from 33 to 90%, with the decrease in acidity from 0.80 to 0.50%.
The content of vitamin C found in the juice (Table 2), at harvest, was low, with respect to values reported in literature for table grapes (Valero et al., 2006; Kevers et al., 2007; Serrano et al., 2006). Since ascorbic acid may be easily oxidized in presence of oxygen by different enzymes, such as ascorbic acid oxidase, phenolase and peroxidase, when cellular disorganization occurs as result of mechanical damage or processing, it was conceivable that during the sample preparation a lowering of the concentration of ascorbic acid occurred. During cold storage a decrease of vitamin C content was observed in almost all the varieties.
‘Crimson’ and ‘Red Globe’, two red grapes varieties, showed the highest total phenolic content at harvest (47,76 and 48.06 mgGAE/100g, respectively), while the level of these components in the ‘Black Pearl’ variety, the other red grape cultivar, was similar to that of the white varieties ‘Sugraone’ and ‘Vittoria’ (Table 2). The lowest total phenolics content was found in the ‘Italia’ variety. Generally, the total phenolic content of red grape is greatly higher than that of white grapes due to the lack of anthocyanins in the skin of the latters (Cantos et al., 2002). However, our results showed that the phenolic content of different grapes depends mainly on the varietal differences, not on grape skin color. As storage advanced, phenolics content decreased. Thus after 7 days, significant differences were observed in ‘Vittoria’, ‘Crimson’, ‘Red Globe’ and ‘Black Pearl’ varieties. At the last stage of storage a further reduction in the phenolic content was recorded in these varieties. Total phenolics of ‘Vittoria’ and ‘Italia’ varieties remained unchanged during 7 days of storage, then a significant decrease was observed at day 15.
All grape varieties studied showed elevated ORAC values ranging from 3156.33 (‘Sugraone’) to 4489.17 μmol TE/100g (‘Red Globe’). During storage a prolonged and significant decrease of TAA was detected for all varieties analyzed. However, at the end of treatment, the antioxidant activity levels in all the genotypes (ranging from 1724,00 – 2861.67 μmol TE/100g) were not such as to lead to an excessive reduction in antioxidant capacity of grapes. A positive correlation ($r = 0.72$, $p<0.001$) between antioxidant activity and total phenolic content, within the genotypes tested was observed. A low but significant correlation ($r = 0.21$, $p < 0.05$) was also found between

antioxidant activity and vitamin C. Since the white varieties are richer in flavan-3-ols than red varieties, the high values of antioxidant activity found also in these genotypes may be related to flavan-3-ols content (Frankel et al., 1998).

With the aim of obtain a better differentiation among the grape varieties and the storage time, some physicochemical parameters of fruit and juice were investigated by multivariate analysis, applying an explorative Linear Discriminant Analysis (LDA). This statistical method may be considered as a dimension reduction technique which allows class separation in a two dimensional plot to be visualized. In this case one or more mathematical functions, referred to canonical discriminant functions, are developed which are simple linear combinations of original variables. If we have k classes (or groups), k-1 canonical functions can be derived. Nine physicochemical parameters (firmness, pH, TSS, fructose, glucose, TA, vitamin C, total phenols, ORAC unit), were chosen as original variables of the statistical analysis. The number of function, obtained by linear combination of original variables, which can be developed is one less than the number of groups. The eigenvalue associated to the first function contributed for 41.7% of the variance of the original data, the eigenvalue associated to the second function contributed for 28.4% of the variance and the eigenvalue associated to the third function contributed for 17.6% of the variance. Therefore, the combination of the first and the second and third functions explain 96.7% of the variance. According to the standardized discriminant function coefficients obtained, fructose (1.773), total phenols (1.593) and glucose (1.496) were the most significant components for differentiation of groups within the first function, and glucose (1.075), vitamin C (0.951) and titratable acidity (0.681) for the second one. It is possible to visualize how these two function discriminate between groups by plotting the individual scores for the two discriminant functions (Fig 1). According to the first function (function 1), 'Vittoria' 'Sugraone' and 'Italia' were well separated, whereas the second function (function 2), gives a clear discrimination among 'Black Pearl', 'Crimson' and 'Red Globe'. In addition, the groups of 'Vittoria' and 'Italia' were separated from 'Crimson' and 'Black Pearl' by second function. Finally, the first function mainly discriminated among white varieties ('Vittoria', 'Sugraone' and 'Italia') and the second function among red varieties ('Black Pearl', 'Crimson' and 'Red Globe'). LDA was also used for differentiation of groups according to the storage time. In this case the eigenvalue associated to the first function contributed for 98.5% of the variance of the original data, the eigenvalue associated to the second function contributed for 1.5% of the variance. The variables with the highest discrimination power for differentiation of storage time were vitamin C (1.146), ORAC (1.096) and TA (0.963). Finally, plotting the

individual scores of the first two functions (Fig. 2) it was noted that the first function discriminated among the three time of storage, while no separation between groups was observed with the second function.

CONCLUSIONS

In conclusion, the results suggest that minimally processed grape stored in air atmosphere maintained the quality attribute during cold storage. No weight loss and browning of the grape berries were observed during cold storage. TSS and TA, important quality factors of table grape for the acceptability by consumers, were not significantly affected during storage. In addition, the significant reduction of TA recorded in 'Vittoria' and 'Crimson' led to, in these genotypes, a increase in the TSS/TA ratio throughout the cold storage. The main sugars found in juices of all table grapes, were glucose and fructose while sucrose occurred at much lower concentrations.

Our results show that significant differences in phytochemical content such as vitamin C and total phenols can exist among grape varieties and that the antioxidant activity in grapes is positively correlated with total phenolic content. These components decreased during cold storage, thus causing the reduction of TAA.

The LDA analysis was used as a potential approach in the discrimination of different varieties and time of storage. Glucose, total phenols and fructose were identified as the most important variables in aiding discrimination among white varieties, while glucose, vitamin C and titratable acidity have differentiated red varieties. In addition, this method was useful to discriminate the groups in relation to time storage.

Literature Cited

AOAC. 1990. Vitamin C (ascorbic acid), 2,6 dichloroindophenol titrimetric method. In K. Helrich (eds.), Official Methods of Analysis, (15th ed.) Arlington Virginia.

Cantos, E., Espin, J. C. and Tomas-Barberan, A. 2002. Varietal differences among the polyphenol profile of seven table grapes cultivars studied by LC-DAD-MS-MS. J. Agric. Food Chem.50:5691-5696.

Carvajal-Millàn E., Carvallo T., Orozco J.A., Martinez M., Tapia I., Guerrero V.M., Chu A.R., Liamas J., Gardea A.A. 2001. Polyphenol oxidase activity, color changes, and dehydration in table grape rachis during development and storage as affected by N-(2-chloro-4-pyridil)-N-phenylurea. J. Agric. Food Chem. 49: 946-951.

Crisosto C.H., Garner D., Crisosto G. 2002. Carbon dioxide-enriched atmospheres during cold storage limit losses from *Botrytis* but accelerate rachis browning of "Redglobe" table grapes. Postharvest Biol. Technol. 26: 181-189.

Frankel, E. N., Bosanek, C. A., Meyer, A. S., Silliman, K. and Kirk, L. L. 1998. Commercial grape juices inhibit the in vitro oxidation of human low-density lipoprotein. J. Agric. Food Chem. 46: 834-838.

Jayasena V. and Cameron I. 2008. °Brix/Acid ratio as a predictor of consumer acceptability of Crimson seedless table grapes. J. Food Quality. 31: 736-750.

Kevers C., Falkowski M., Tabart J., Defraigne J. O., Dommes J. and Pincemail J. 2007. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J. Agric. Food Chem. 55: 8596-8603.

Lovino R., Massignan L., De Cillis F.M., Santomasi F., Liturri D. 2003. Uva da tavola di IV gamma. L'Informatore Agrario. 49: 1-3.

Ou, B., Hampsch-Woodill, M. and Prior, R. 2001. Developing and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry, 49: 4619–4626.

Piva C.R., Lopez Garcia J.L., Morgan W. 2006. The ideal table grapes for the Spanish market. Rev. Bras. Frutic. 28 n.2: 258-261.

Serrano M., Valverde J. M. , Gillen F., Castillo S., Martinez-Romero D. and Valero D. 2006. Use of *Aloe vera* gel coating preserves the functional properties of table grapes. J. Agric. Food Chem. 54: 3882-3886.

Singleton V. L., Orthofer R. and Lamuela-Raventos R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods in Enzimology. 299: 152-178.

Valero D., Valverde J. M., Martinez-Romero D., Guillen F., Castillo S. and Serrano M. 2006. The combination of modified atmosphere packaging with eugenol or thymol to mantain quality, safety and functional properties of table grapes. Postharvest Biol. Technol. 41:317-327.

TABLES

Table 1 – Change of quality attribute of different grape varieties during cold storage[(a)]

Grapes varieties		T0	T7	T15
Firmness (g)	Sugraone	900.00	845.46	805.93
	Vittoria	660.52	646.07	637.45
	Italia	511.93A	436.93B	347.80C
	Crimson	932.12	888.27	874.52
	Red Globe	757.93A	736.48AB	660.02B
	Black Pearl	932.12A	909.71AB	847.83B
pH	Sugraone	4.00	4.00	4.05
	Vittoria	3.97	3.97	3.88
	Italia	4.02	3.96	3.93
	Crimson	3.89	3.96	3.92
	Red Globe	4.16	4.23	4.08
	Black Pearl	4.04	3.99	4.10
TSS (%)	Sugraone	17.68	17.80	18.23
	Vittoria	14.54	13.68	13.89
	Italia	20.18	21.44	19.57
	Crimson	17.87	15.37	17.14
	Red Globe	20.17A	17.37B	17.75B
	Black Pearl	20.65	19.94	20.44
Fructose (g/100mL)	Sugraone	8.58	8.92	8.99
	Vittoria	6.65AB	6.52B	7.01A
	Italia	10.35	10.73	10.38
	Crimson	9.96A	8.93B	10.36A
	Red Globe	11.80A	8.52C	9.26B
	Black Pearl	10.96A	9.79B	10.11B
Glucose (g/100mL)	Sugraone	7.86	7.84	8.15
	Vittoria	7.23	6.84	7.08
	Italia	9.46	9.67	9.17
	Crimson	9.79A	8.39B	9.95A
	Red Globe	10.38A	7.71B	8.10B
	Black Pearl	10.96A	9.88B	10.02B
TA (g/100mL tartaric acid)	Sugraone	0.60	0.63	0.58
	Vittoria	0.48A	0.49A	0.45B
	Italia	0.56	0.59	0.58
	Crimson	0.64A	0.53B	0.57B
	Red Globe	0.42	0.44	0.48
	Black Pearl	0.67	0.66	0.63

[(a)]Means in the same row followed by different letters are significantly different ($p < 0.01$); no letters, not significant

Table 2- Change of antioxidant components and ORAC units in different grape varieties during cold storage[(a)]

Grapes varieties		T0	T7	T15
Vitamin C (mg/100 mL)	Sugraone	5.52A	3.51B	2.51B
	Vittoria	4.68A	3.84A	2.67B
	Italia	9.15A	8.07B	5.37C
	Crimson	5.74A	1.82B	1.49B
	Red Globe	5.45A	1.90B	1.65B
	Black Pearl	1.82	1.73	1.73
Total phenolics (mg/100g)	Sugraone	36.44A	36.22A	29.38B
	Vittoria	36.97A	33.19B	31.54C
	Italia	21.90A	22.29A	17.51B
	Crimson	47.76A	29.89B	20.06C
	Red Globe	48.06A	26.61B	17.19C
	Black Pearl	36.81A	26.99B	19.40C
ORAC units (µmoli trolox equiv./100 g)	Sugraone	3156.33A	2495.33B	2098.00C
	Vittoria	3934.00A	3295.33B	2861.67C
	Italia	3303.00A	2489.67B	2064.67C
	Crimson	3849.67A	2710.67B	1724.00C
	Red Globe	4489.17A	3284.33B	2753.33C
	Black Pearl	4363.67A	3382.00B	1778.00C

[(a)]Means in the same row followed by different letters are significantly different ($p < 0.01$); no letters, not significant

FIGURES

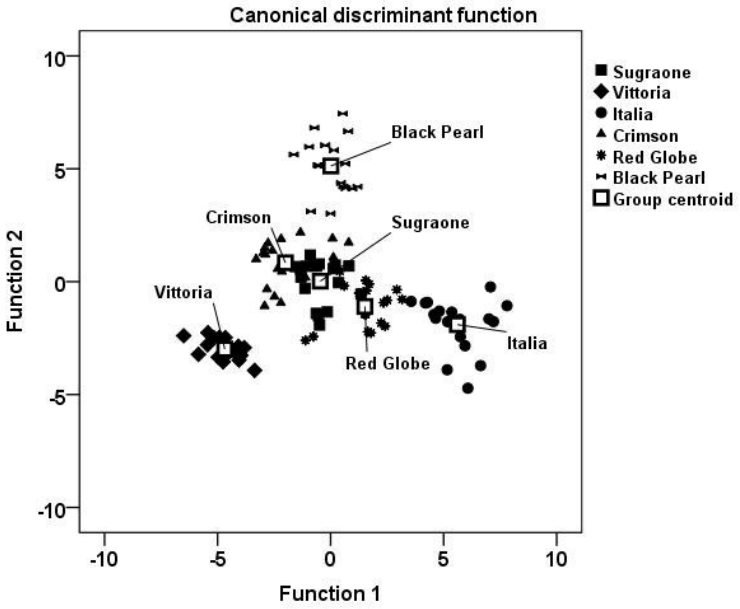

Figure 1 – Plot of the two discriminant function scores for the grape varieties

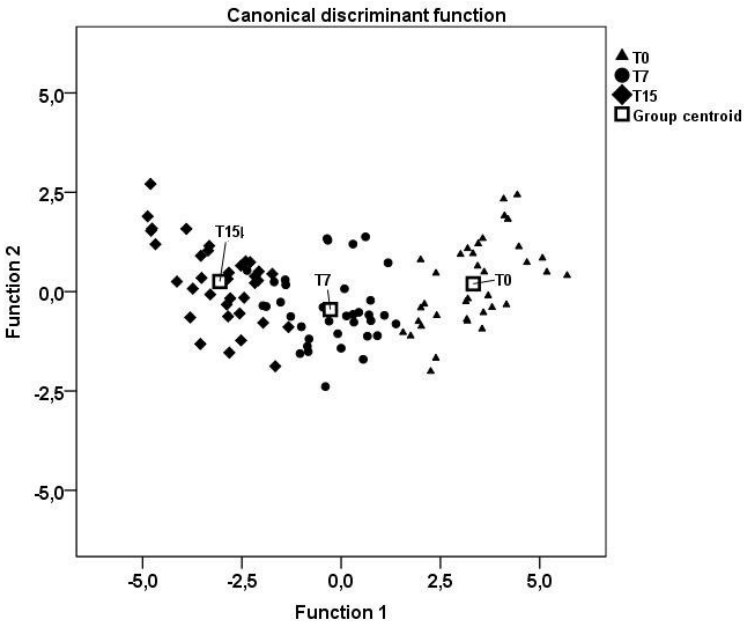

Figure 2 – Plot of the two discriminant function scores for the time storage

PUBLI.7

Innovative Food Science and Emerging Technologies 11 (2010) 477–484

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Supercritical carbon dioxide-treated blood orange juice as a new product in the fresh fruit juice market

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ARTICLE INFO

Article history:
Received 28 December 2009
Accepted 28 February 2010

Editor Proof Receive Date 30 March 2010

Keywords:
Continuous process
Fresh blood orange juice
High-pressure CO_2
Antioxidants
Microbial survival
Sensory attributes

ABSTRACT

The work described here deals with the effectiveness of using high-pressure carbon dioxide treatment (HPCD) to stabilise freshly squeezed blood orange juice. Technical planning of a continuous high-pressure supercritical carbon dioxide pilot system, suitable for development on an industrial scale, was carried out in our lab. To determine the optimal operating conditions (temperature, pressure, and CO_2/juice ratio), three different experimental trials were carried out. The first trial was conducted at 230 bar, 36 ± 1 °C, 5.08 L/h juice flow rate, and 3.91 L/h CO_2 flow rate, corresponding to a g_{CO2}/g_{juice} ratio of 0.770. The second trial utilised the same conditions except that the operative pressure was reduced (130 bar). The third trial was carried out at 130 bar, 36 ± 1 °C, 5.08 L/h juice flow rate, 1.96 L/h CO_2 flow rate, corresponding to a 0.385 g_{CO2}/g_{juice} ratio. The effects of processing were evaluated by determining physicochemical, antioxidant, and microbiological parameters of the treated juices. In addition, once the best operative parameters had been determined, physicochemical, antioxidant, microbiological and sensory evaluation of fresh blood orange juice stabilised by HPCD treatment was carried out during refrigerated storage of juices at 4 ± 1 °C for thirty days. The results showed that HPCD treatment cannot be considered as an alternative to traditional thermal methods but as a new mild technology for producing a stabilised blood orange juice with a shelf-life of 20 days.

Industrial relevance: Blood oranges are the main cultivated varieties of *Citrus sinensis* (L.) Osbeck in Italy. Freshly squeezed blood orange juice exert a high antiradical and antioxidant activity, due to its rich phenolic profile, but its preservation is usually assured by thermal treatment which affects its nutritional and sensory value. In this study we proposed a "milder" continuous HPCD process suitable for implementation on an industrial scale. The HPCD stabilised juice retains its physicochemical, antioxidant, and sensory properties and could be placed within a new retail framework, namely, that of fresh juices with a shelf-life of 20 days.

1. Introduction

Thermal food preservation is an established and well-known technique used to stabilise foods. Although pasteurisation is very effective for the microbiological stabilisation of foods and inactivating pectin methyl esterase (PME) in fruit juices (Marcotte, Stewart, & Fustier, 1998; Shomer, Cogan, & Mannheim, 1994), this treatment affects the intrinsic quality of a majority of food products with regards to their nutritional and organoleptic value, especially for fruit juices (Shomer et al., 1994). Due to the increase in consumer demand for nutritious, fresh-like food products with a high organoleptic quality and an extended shelf-life, non-thermal processing alternatives have been proposed. Among these non-thermal inactivation technologies, high hydrostatic pressure (HHP) and pulsed electric fields (PEF) are the most investigated ones (Devlieghere, Vermeiren, & Debevere, 2004; Jeyamkondan, Jayas, & Holley, 1999). Although HHP offers great opportunities for food preservation, it also entails a large investment of capital due to the high pressures involved. Efforts to use the PEF method on a commercial scale for the pasteurisation of food have resulted in at least two industrial-scale PEF systems, including treatment chambers and power supply equipment (Butz & Tauscher, 2002). However, PEF technology has not yet attained the commercial usability stage (Gerlach et al., 2008), while the high capital cost of such systems remains a major obstacle to its industrial application.

The use of high-pressure carbon dioxide (HPCD) has been proposed as an alternative non-thermal pasteurisation technique for foods (Spilimbergo, Elvassore, & Bertucco, 2003; Kincal, Hill, Balaban, Marshall, & Wei, 2005; Boff, Truong, Min, & Shellhammer, 2003). Many conflicting hypotheses regarding microbial inactivation by HPCD can be found in the literature (Nakamura, Enomoto, Fukushima, Nagai, & Hakoda, 1994; Enomoto, Nakamura, Nagai, Hashimoto, & Hakoda, 1997; Dillow, Denghani, Hrkack, Foster, & Langer, 1999). However, the exact inactivation mechanisms remain unknown. Recently, Garcia-Gonzalez et al. (2007) proposed a model for how

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doi:10.1016/j.ifset.2010.02.004

pressurised CO_2 may exert its lethal action on bacteria. This model consists of the following steps: (I) solubilisation of pressurised CO_2 in the external liquid phase; (II) cell membrane modification, (III) intracellular pH decrease; (IV) key enzyme inactivation/inhibition of cellular metabolism due to decreased intracellular pH; (V) inhibitory effect of molecular CO_2 and HCO_3^- on metabolism; (VI) disordering of the intracellular electrolyte balance; and (VII) removal of vital constituents from cells and cell membranes, which, according to the authors, take place simultaneously and not one after the other.

HPCD treatments can be conducted in a batch, semi-batch, or continuous manner. In a batch system, CO_2 and treatment solution are stationary in a vessel, while a semi-continuous system involves a continuous flow of CO_2 through a chamber. Early studies employed batch systems that were not suitable for industrial development of the HPCD technology (Ballestra, Abreu Da Silva, & Cuq, 1996). A semi-continuous system was developed by Ishikawa, Shimoda, Shiratsuchi, and Osajima (1995). This system involved the use of a stainless steel mesh filter to produce CO_2 micro-bubbles, which was attached to the bottom of a pressure vessel. They showed that this system could achieve three times more enzyme inactivation than without the micropore filter. However, the commercial application of batch or semi-continuous HPCD treatments has been limited because of their low processing capacity. In 1999, Praxair (Chicago, Ill., U.S.A.) developed a high-pressure continuous flow HPCD system. In this system, both CO_2 and product are pumped through the system and mixed before passing through a high-pressure pump, which increases the pressure to the required processing levels. At the end of the process, an expansion valve is used to release CO_2 from the mixture. This system has been shown to be very effective at killing pathogens and spoilage bacteria in a short time (Kincal et al., 2005) and has been proposed under the trademark "Better Than Fresh (BTF)". However, this equipment found no commercial success, probably due to the high cost of the system, whose operative pressure values can even reach 1100 bar. Parton, Bertucco, Elvassore, and Grimolizzi (2007) presented a continuous plant designed for microbial inactivation by high-pressure CO_2 treatment under milder conditions (80–110 bar). The authors obtained encouraging results with both simple and complex pumpable foodstuffs, even under mild conditions. However, this continuous plant was designed for processing only 1 L/h of liquid.

Most of the published work in the literature thus far focuses on microbial inactivation immediately after HPCD treatment. Little information is available about the effects on the sensory, nutritional, and antioxidant properties of liquid foods after HPCD treatment and also on long-term microbial stability in HPCD-treated foods during refrigerated storage.

Blood oranges, including Tarocco, Moro, and Sanguinello, are the most widely processed varieties in the Italian citrus juice industry. Blood orange juice is characterised by the presence of anthocyanins, which are responsible for its red colour, along with the high content of other antioxidant compounds such as vitamin C, flavanones, and hydroxycinnamic acids (Rapisarda, Bellomo, & Intelisano, 2001). Previous studies have shown that the antioxidant activity of blood orange juice was higher than that found in blond or common orange juice and that antioxidant efficiency appears to be correlated with anthocyanins level (Rapisarda et al., 1999). In addition, blood orange juice consumption improved the resistance of lymphocyte DNA to oxidation stress and the antioxidant defences of organisms (Riso et al., 2005).

The aim of this work was to evaluate the effects of HPCD treatment on the physicochemical, enzymatic (looking specifically at PME), and antioxidant parameters of freshly squeezed blood orange juice treated, under different operative conditions, using a "milder" continuous process that is suitable for implementation on an industrial scale for pumpable liquid substrates. The effects of HPCD processing were even compared with those produced by traditional thermal treatment. Moreover, a study was performed to evaluate the effect of the HPCD treatments on bacterial growth (mesophilic viable counts, yeasts and moulds, and spoilage microorganisms of citrus products) during refrigerated storage of juices at 4 ± 1 °C for thirty days.

Three experimental trials were carried out in order to identify more suitable operating conditions for the industrial scaling-up of cold-pasteurisation technology. Finally, once the best operative parameters to be employed were determined, physicochemical, antioxidant and sensory evaluation of fresh blood orange juice stabilised by HPCD treatment was carried out during refrigerated storage of juices at 4 ± 1 °C for thirty days.

2. Materials and methods

2.1. High-pressure supercritical CO_2 equipment

The equipment was designed in our laboratory according to European Commission regulations for pressure systems (PED — Pressure Equipment Directory). It consists of two parts: a continuous HPCD system and a batch high-pressure carbon dioxide extractor. This equipment was entirely realised in AISI 316 L steel, by Separeco s.r.l. (Pinerolo, TO, Italy). In this work, only the continuous high-pressure equipment was used. The whole system is polyurethane-insulated to prevent heat loss. Pumps were designed to resist pressures of up to 330 bar before security valves are opened. Fig. 1 shows a scheme of the plant. The T1 tank (containing orange juice or, alternatively, cleaning solutions) is connected to the PO2 pump (Lewa, max flow 5.08 L/h). CO_2 (99.95% grade purity) is subcooled in a cooling system (CS) in order to be pumped as a liquid by the PO1 pump (Lewa, max flow 5.87 L/h). The supercritical CO_2 and the processed liquid become pressurised independently, and their flows converge when they reach the mixer (M1) after opening valve VM1. After being mixed through four mixing lines (mixer M1), the CO_2–juice mixture reaches the holding tube (P1). A temperature probe (TP1) is connected, and the mixer temperature value is displayed on the electric panel. The holding tube is about 10 m in length and consists of 12 connected pipes, each with an external diameter of ½ in. Hot water from a thermostatic bath flowing in external pipes allows the whole holding tube to be thermostated. Six temperature probes (TP2–TP7) are arranged along the holding tube for temperature control. At the holding tube outlet, a laminating valve (VL3) allows depressurisation of the plant. In order to avoid freezing problems due to the isoenthalpic expansion of the CO_2, the laminating valve is covered with an electric resistance. After depressurisation, the CO_2–juice mixture reaches a tank (B1) that is connected to a smaller tank (B2), which is in turn connected to a vacuum pump (PO3) for degassing. The amount of CO_2 employed for processing is measured with a gas counter (GC).

2.2. Processing and cleaning procedures

For each treatment, at least 10 L of freshly squeezed blood orange juice were used, while an amount was used as control sample for analysis. The orange juice feed tank (T1) and the collecting tank (B1) were sterilised by autoclaving before processing. At the end of each treatment, tank B1 was disconnected and then opened to get samples for analysis, taking care to maintain sterility. Before processing and at the end of each trial a CIP (cleaning in place) practice was adopted. This procedure consisted of six consecutive cycles, including distilled water for preliminary washing, 5% w/w aqueous solution of NaOH to eliminate crustings, distilled water again for rinsing, and finally, a 3:10 v/v aqueous solution of hypochlorite. At the end of the cleaning steps, distilled and then sterile water were flushed through the plant.

Three experimental trials were carried out under different operative conditions. The first trial (treatment 1) was conducted at

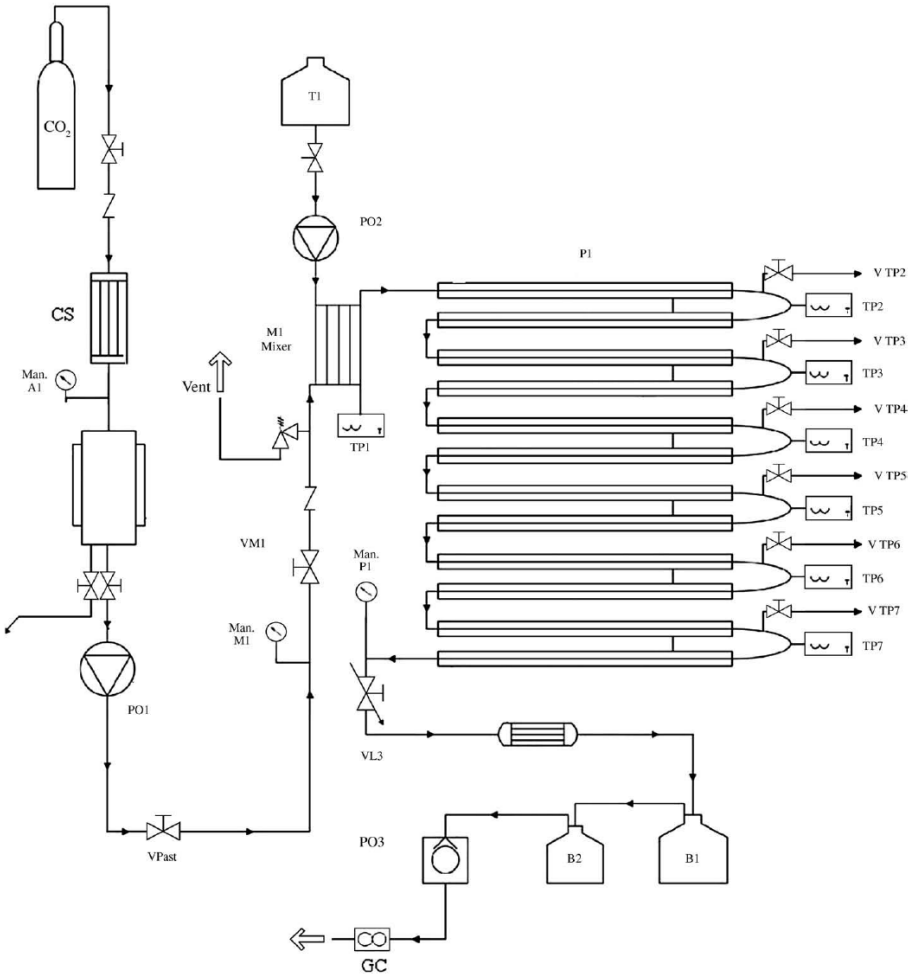

Fig. 1. Scheme of the continuous pilot plant for HPCD pasteurisation of freshly squeezed blood orange juice.

230 bar, 36 ± 1 °C, 5.08 L/h juice flow rate, and 3.91 L/h of CO_2 flow rate, corresponding to 0.770 g_{CO2}/g_{juice}. The second trial (treatment 2) used the same conditions except that the operative pressure was reduced (130 bar). The third trial (treatment 3) was carried out at 130 bar, 36 ± 1 °C, 5.08 L/h juice flow rate, and 1.96 L/h CO_2 flow rate, corresponding to 0.385 g_{CO2}/g_{juice}. In all three trials, the temperature was set in order to ensure supercritical CO_2 conditions. The residence time was 15 min for each trial. To evaluate the effectiveness of the process, an amount of freshly squeezed blood orange juice was thermally pasteurised at 88–91 °C in a tubular heat exchanger for 30 s for each trial and then analysed together with the HPCD-treated orange juice samples.

2.3. Physicochemical analysis

Titratable acidity (TA), total soluble solids (TSS), and pH were determined according to conventional methods (Kimbal, 1999). Colour analysis was evaluated as CIE $L^*a^*b^*$ values, using CIE D65 as illuminant, by recording the percentage of reflectance from 830 to 360 nm (Varian UV–Vis spectrophotometer model Cary 100 Scan).

PME activity was measured titrimetrically at pH 7.0 and 25 °C using the method of Rouse and Atkins (1955) and expressed as μmol carboxyl released per min per mL of sample. Cloud was measured by recording the absorbance at 660 nm (Kincal et al., 2006).

2.4. Antioxidant components

The ascorbic acid concentration was evaluated by liquid chromatography using a Waters Alliance 2695 HPLC equipped with a Waters 996 photodiode array detector and Empower software (Rapisarda & Intelisano, 1996). Total anthocyanin content was determined spectrophotometrically (Varian UV–Vis spectrophotometer model Cary 100 Scan) by the pH differential method (Rapisarda, Fanella, & Maccarone, 2000). Flavanone glycosides concentration, expressed as hesperidin equivalents (mg/L), were determined by HPLC (Rouseff, Martin, & Youtsey, 1987). Samples were analysed for total phenolics by Folin–Ciocalteu (FC) colourimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Antioxidant activity was determined using the ORAC assay, as described by Ou, Hampsch-Woodill, and Prior (2001), with some modifications. Briefly, the measurements were carried out on a Wallac 1420 Victor III 96-well plate reader (EG & Wallac, Turku, Finland) with a fluorescence filter (excitation 485 nm, emission 535 nm). Fluorescein (116 nM) was the target molecule for free radical attack from AAPH (153 mM) that was used as the peroxyl radical generator. The reaction was conducted at 37 °C and pH 7.0, with Trolox (10 μM) as the control standard and 75 mM phosphate buffer (pH 7.0) as the blank. All solutions were freshly prepared prior to analysis. The samples were diluted with phosphate buffer (1:25–100, v/v) prior to analysis, and results were reported as micromoles of Trolox equivalents per 100 mL of juice.

2.5. Microbial analysis

Microbiological analyses were performed at time 0, the same day of the HPCD treatment, and every 5 days thereafter for a period of 30 days. Untreated juice was analysed on the day of the treatment as control. Viable cells in treated and untreated juices were counted by using solid growth medium and serial decimal dilutions in sterile physiological saline solution. Mesophilic viable count (MVC) was performed on Plate Count Agar (PCA, Oxoid, CM325), after 48 h incubation at 32 °C. Yeast and mould counts were determined with Sabouraud Dextrose Agar (SAB, Oxoid, CM41) after incubation at 25 °C for 2–4 days. Spoilage microorganisms that typically contaminate orange juice were counted on Orange Serum Agar (OSA, Oxoid, CM0657) plates incubated at 30 °C and examined after 2–4 days.

2.6. Sensory analysis

The profile method (ISO 13299, 2003) was used for the sensory evaluation of HPCD-treated blood orange juice processed at 130 bar, 36 ± 1 °C, 0.385 g_{CO2}/g_{juice} (treatment 3). This method involves the quali-quantitative description of the sample sensory attributes made by a trained panel (ISO 8586-1, 1993) in a laboratory organised within CRA-ACM. Twenty panelists, male and female, aged between 28 and 45 years old, were selected among the staff of CRA-ACM. In a preliminary session, the judges selected the attributes to describe the juice (colour, freshness, intensity of the scent, acidity, sweetness, flavour, off-flavour, bitterness, and intensity of taste). In subsequent sessions, the judges evaluated the intensity of each chosen attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense) on a numerical unipolar scale. Sensory analyses were carried out at time 0 the same day of the treatment and every 5 days thereafter for a total of 30 days of refrigerated storage of juice at 4 ± 1 °C.

2.7. Statistical analysis

Statistical elaboration of the results was carried out with the program STATSOFT 6.0 (Vigonza, Padova, Italy). The statistical differences between UJ (untreated juice), TJ_{CO2} (HPCD-treated juice) and TJ_{TP} (thermally pasteurised juice) were evaluated for each treatment by variance analysis (ANOVA), and the means separation was executed by Tukey test. One-way ANOVA and the Tukey test were also employed to determine differences in sensory attributes of juice during the 30 days of refrigerated storage at 4 ± 1 °C.

3. Results and discussion

3.1. Physicochemical analysis

Table 1 shows TSS, TA, and pH values of UJ, TJ_{CO2}, and TJ_{TP} obtained with the three different tests. No statistically significant differences between treated and control samples were found for these parameters. The same results were reported by Balaban et al. (1991) for a static supercritical CO_2 system and Kincal et al. (2006) for a continuous HPCD system. With regards to colour parameters, the L^*, a^*, and b^* values decreased after CO_2 treatment. However, these changes were not drastic enough to significantly alter the characteristic colour of the juice.

Cloud is a significant quality attribute of orange juice that contributes to its flavour, aroma and turbidity (Baker & Cameron, 1999). Cloud loss has been attributed to the enzymatic activity of PME, which is responsible of the de-esterification of pectins (Boff et al., 2003). Consumers usually associate cloud loss with spoilage and quality loss of orange juices. Hence, maintenance of cloud is important to the overall quality of the product. In the present study, TJ_{TP} had only 0.82% average residual PME activity (Fig. 2), in accordance with what was found by Boff et al. (2003), and an average cloud increase of 56.58% (Fig. 3). However, TJ_{CO2} samples showed a lower inactivation of PME, with an average percentage remaining activity of 66.81%, 59.12%, and 55.84% respectively for treatments 1, 2, and 3 (Fig. 2). As demonstrated by Goodner, Braddock, and Parish (1998), a portion of PME can be inactivated easily by pressure, but some PME isozyme remains active even after strong pressurisation. Although PME was still active, cloud values of TJ_{CO2} were much higher with respect to UJ and TJ_{TP}. Average values for cloud increase, with respect to UJ, of 263.27%, 109.59% and 162.20% were achieved for treatments 1, 2 and 3, respectively, indicating that the pressure applied for the CO_2 treatments has an influence on cloud (Fig. 3). Therefore, cloud was not only preserved but also enhanced. This phenomenon is probably due, as other authors have previously supposed (Kincal et al., 2006), to the depressurisation of the system, which homogenises orange juice,

Table 1
Physicochemical parameters in UJ (untreated juice), TJ_{CO2} (treatments 1, 2, 3) and TJ_{TP} (thermally pasteurised treated juice). Means in the same row followed by different letters are significantly different: $p \leq 0.01$; $n = 3$ processing runs.

	TSS (°Brix)	pH	TA (% citric acid)	L^*	a^*	b^*
Treatment 1						
UJ	12.88	3.39	1.09	31.22 A	30.58 A	25.10 a
TJ_{CO2}	12.34	3.44	1.09	28.33 B	26.27 B	22.46 b
TJ_{TP}	12.52	3.43	1.04	30.33 AB	29.02 A	24.89 a
Treatment 2						
UJ	12.59	3.56	1.12	34.54 A	32.02 A	30.18 A
TJ_{CO2}	12.69	3.53	1.13	30.80 B	27.41 B	25.02 B
TJ_{TP}	11.89	3.57	1.01	32.28 B	28.08 B	25.92 B
Treatment 3						
UJ	10.68	3.42	1.09	32.03 A	30.32 A	28.60 A
TJ_{CO2}	10.69	3.43	1.02	26.43 B	24.17 C	20.11 B
TJ_{TP}	10.63	3.44	1.11	31.79 A	28.21 B	26.65 A

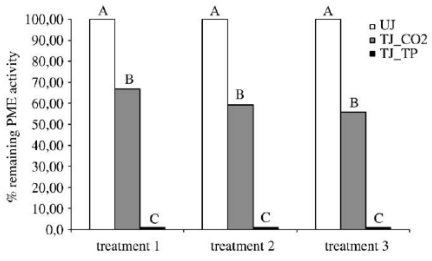

Fig. 2. Pectinesterase activity in UJ (untreated juice), TJ_{CO2} (treatments 1, 2 and 3) and TJ_{TP} (thermally pasteurised treated juice). Means signed by different letters are significantly different ($p \leq 0.01$).

causing the rupture of particles in the juice colloid, thereby increasing cloud.

3.2. Changes in antioxidant components

A retention of antioxidant compounds was observed for TJ_{CO2} juices processed at lower pressure (treatments 2 and 3), as shown in Table 2. The concentration of CO_2 applied during HPCD processing did not significantly affect antioxidant retention. Thermal pasteurisation was more detrimental to antioxidant components than HPCD treatment, whereas for treatment 1, no difference was noted between TJ_{CO2} and TJ_{TP}.

The average vitamin C content was reduced after treatment 1 but remained unchanged, with respect to UJ, after the treatments at lower pressure, regardless of the amount of CO_2 employed. Ascorbic acid degradation is characterised by simultaneous aerobic and anaerobic reactions, with aerobic degradation being the fastest (Ahrne, Manso, Shah, Oliveira, & Oste, 1996). The addition of CO_2 to orange juice is probably beneficial to ascorbic acid retention due to the displacement of dissolved oxygen from the liquid matrix (Boff et al., 2003). Moreover, lower operative pressures are probably beneficial for the retention of this antioxidant component.

Changes in total anthocyanin content after treatment 1 were in line with the trend seen for vitamin C. No significant differences were observed between UJ and TJ_{CO2} for either treatments 2 and 3. Losses in total flavanones, with respect to UJ, were seen in the TJ_{TP} juices from treatments 1 and 3. Moreover, total flavanones were slightly reduced after HPCD treatment 1, remaining unchanged after HPCD treatments 2 and 3. The total phenolic content decreased significantly after all thermal treatments and HPCD treatment 1 but remained constant

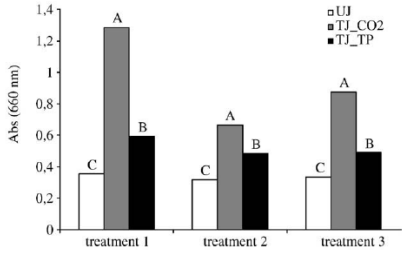

Fig. 3. Cloud values in UJ (untreated juice), TJ_{CO2} (treatments 1, 2 and 3) and TJ_{TP} (thermally pasteurised treated juice). Means signed by different letters are significantly different ($p \leq 0.01$).

Table 2
Changes in antioxidant components and ORAC units in UJ (untreated juice), TJ_{CO2} (treatments 1, 2, 3) and TJ_{TP} (thermally pasteurised treated juice). Means in the same row followed by different letters are significantly different: $p \leq 0.01$ — capital letter; $p \leq 0.05$ — small letter; $n = 3$ processing runs.

	UJ	TJ_{CO2}	TJ_{TP}
Treatment 1			
Vitamin C (mg/100 mL)	63.66 A	59.60 B	61.98 AB
Total anthocyanins (mg/L)	68.47 A	62.87 B	64.92 AB
Total flavanones (mg/L)	109.96 A	102.40 B	104.79 B
Total phenolics (mg/L)	1229.48 A	1093.53 B	1065.95 B
ORAC units (μM trolox equiv./100 mL)	2662.33 a	2625.21 a	2515.98 b
Treatment 2			
Vitamin C (mg/100 mL)	63.01	63.29	58.05
Total anthocyanins (mg/L)	66.74 a	65.33 a	59.27 b
Total flavanones (mg/L)	103.20	103.47	101.43
Total phenolics (mg/L)	1226.99 A	1122.99 A	1049.67 B
ORAC units (μM trolox equiv./100 mL)	3622.94 A	3531.33 A	3228.49 B
Treatment 3			
Vitamin C (mg/100 mL)	61.20 a	61.17 a	59.15 b
Total anthocyanins (mg/L)	70.06 a	69.32 a	66.60 b
Total flavanones (mg/L)	113.91 a	106.86 ab	103.12 b
Total phenolics (mg/L)	1167.32 a	1105.82 a	1050.72 b
ORAC units (μM trolox equiv./100 mL)	2984.00 A	3032.63 A	2764.28 B

after HPCD treatments 2 and 3. Anthocyanin and phenolic degradation in thermally pasteurised orange juices is presumably due to the formation of carbohydrate and organic acid degradation products during thermal processing, such as furfurals and other carbonyl compounds, which can form condensation products with anthocyanins and polyphenols (Del Pozo-Insfran, Balaban, & Talcott, 2006). Even HPCD treatment at higher pressure (treatment 1) caused losses in antioxidant components, albeit without affecting total antioxidant capacity. Corrales, Butz, and Tauscher (2008) found that combined temperature/high-pressure treatments may enhance degradation and formation of condensation products in wines, which contribute to colour, organoleptic and nutritional changes, even without a sensible loss of antioxidant capacity. Model systems containing anthocyanins have demonstrated that the degradation of these phytochemicals takes place under both aerobic and anaerobic conditions (Garzon, Wrolstad, & Durst, 2002; Hrazdina, Borzell, & Robinson, 1970). Therefore, the exclusion of oxygen during processing would not be sufficient by itself to prevent such degradation. By this way, it may be hypothesised that HPCD treatment at lower pressure prevents and/or reduces furfurals formation during processing. Thus, HPCD could be used as a useful system to reduce the degradation of polyphenolic compounds. The results of antioxidant activity assay confirm these findings. In fact, ORAC units showed no statistical differences between UJ and TJ_{CO2} for each treatment. This demonstrates that the total antioxidant activity of orange juice is not significantly prejudiced by carbon dioxide treatment. In contrast, thermal pasteurisation was more detrimental compared with HPCD and unprocessed orange juices. Antioxidant capacity was retained for HPCD-processed juices, independent of the g_{CO2}/g_{juice} ratio and the pressures applied, showing again a positive influence of this new technology on the stability of antioxidant compounds of blood orange juice.

3.3. Microbial analysis

Figs. 4–6 show the microbial stability in HPCD-treated blood orange juice processed under different operative conditions, determined at time 0 and during refrigerated storage for 30 days at 4 ± 1 °C. This temperature was chosen to emulate refrigerated storage during retail and household storage. The initial microbial load of freshly squeezed blood orange juice was 8.60×10^3 CFU/mL in SAB, 3.82×10^3 CFU/mL in PCA and 1.09×10^4 CFU/mL in OSA, before treatment 1. With regard to bacterial inactivation achieved by treatment 2, the initial microbial load

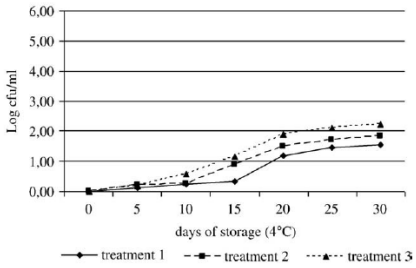

Fig. 4. Yeasts and moulds growth in TJ_{CO2} (treatments 1, 2 and 3) on SAB agar solid plates, during refrigerated storage (4 °C).

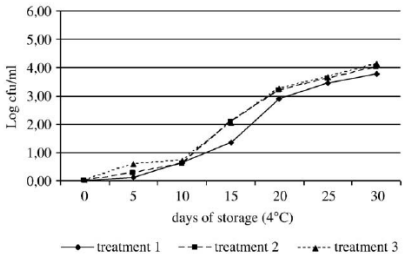

Fig. 6. Orange juice spoilage microorganisms growth in TJ_{CO2} (treatments 1, 2 and 3) on Orange Serum Agar (OSA) solid plates during refrigerated storage (4 °C).

before juice processing was 3.21×10^4 CFU/mL in SAB, 3.31×10^4 CFU/mL in PCA and 2.79×10^4 CFU/mL in OSA. In the last experimental trial (treatment 3), the initial microbial load was 5.68×10^3 CFU/mL in SAB, 2.90×10^3 CFU/mL in PCA and 2.90×10^3 CFU/mL in OSA. Fig. 4 shows the effectiveness of HPCD treatments on yeast and mould inactivation (SAB agar plates). At time 0, immediately after HPCD treatments, there were no culturable organisms present in the juice. As storage continued, counts began to increase. Treatment at higher pressure (treatment 1) produced a better stabilisation, with a microbial load of 1.54 Log CFU/mL after 30 days of storage. Anyway, counts reached acceptable values of 1.81 and 2.23 Log CFU/mL for treatments 2 and 3, respectively. The microbial value of 6 Log CFU/mL was considered unacceptable for juice quality (Kincal et al., 2005).

Fig. 5 shows the influence of HPCD treatments on mesophilic viable counts of freshly squeezed blood orange juice, again indicating a positive influence of operative pressure on bacterial inactivation. However, even treatments at lower operative pressure (130 bar) produced an efficient and long-lasting stabilisation. After 30 days of storage, counts reached values of 4.68 and 4.84 Log CFU/mL, respectively, for treatments 2 and 3, well below the acceptability threshold of 6.0 Log CFU/mL. The changes in the count values during refrigerated storage of microorganisms typically encountered in orange juice, as enumerated on OSA plates, are shown in Fig. 6. Once again, immediately after HPCD treatment, there were no culturable microbial cells present in the juice. Counts began to significantly increase after 15 days, reaching the highest value at the last stage of storage (3.77, 4.01, and 4.11 Log CFU/mL respectively for treatments 1, 2, and 3). Similar behaviour was recorded by Kincal et al. (2005) in orange juice treated with continuous high-pressure CO_2 at 107 MPa, a CO_2/juice ratio of 1.03, and a residence time of 10 min. Microorganisms can exist in a state where they are viable but non-culturable (VNC). Transformation from the vegetative to the quiescent

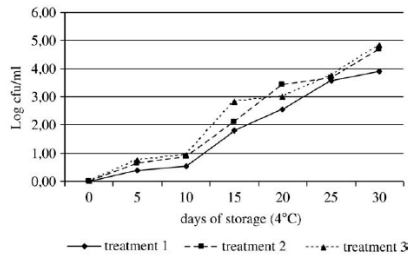

Fig. 5. Mesophilic viable bacteria growth in TJ_{CO2} (treatments 1, 2 and 3) on plate count agar solid plates during refrigerated storage (4 °C).

state is a survival strategy. In this case, we believe that injury takes place during HPCD treatment, thus causing the transformation of vegetative microbial cells to a VNC state. Subsequently, during refrigerated storage, microbial cells repair, and microorganisms revert to their vegetative and culturable state.

In all experimental trials, the results obtained provide evidence that microbial inactivation obtained with the reduced amount of carbon dioxide (treatment 3) was lower than that achieved after the treatment at a higher ratio (treatments 1 and 2). However, freshly squeezed blood orange juice treated under milder conditions in treatment 3, which is more cost effective than the other treatments, resulted in acceptable microbial levels even after 30 days of refrigerated storage.

3.4. Physicochemical changes during refrigerated storage

Analysis were performed every 5 days for a storage period of 30 days (4 ± 1 °C) on TJ_{CO2} (treatment 3) processed using settings that we assumed to be the most favourable for industrial-scale development. Results showed no changes in TSS, TA, pH, colour parameters and PE activity after 30 days of refrigerated storage. Besides, cloud values remained unchanged regardless of storage time (data not shown).

3.5. Changes in antioxidant components during refrigerated storage

Table 3 shows the evolution of antioxidant components in TJ_{CO2} (treatment 3) during refrigerated storage (4 ± 1 °C). Significant differences in vitamin C and total anthocyanin contents were observed only after 25 days of refrigerated storage. Total flavanones and total phenolic contents showed a significant decrease after 15 days. Total antioxidant activity values reflected this trend. ORAC units decreased significantly after 15 days (10.23%), recording a further loss after 20 days (11.84%), then remaining unchanged until the 30th day of refrigerated storage. Anyway, these changes are not so drastic to determine a relevant alteration of antioxidant properties of blood orange juice. Based on their experimental evidences, Prior and Cao suggested that the daily intake of antioxidants should be between 3.000 and 5.000 ORAC units to have a significant impact on plasma and tissue antioxidant capacity (Prior, Joseph, Cao, & Shukitt-Hale, 1999). As a matter of fact, the consumption of two glasses (≈200 mL) of HPCD-treated blood orange juice ensures a high antioxidant protection.

3.6. Sensory evaluation during refrigerated storage

Table 4 shows changes in sensory attributes in TJ_{CO2} (treatment 3) obtained applying processing parameters that we consider suitable for the industrial scaling-up of the new technology. The results are reported as the means of three different sessions carried out after 0, 5,

Table 3
Changes in antioxidant components and ORAC units in TJ_{CO2} (treatment 3) during refrigerated storage at 4 °C (30 days). Means signed by different letters are significantly different ($p \leq 0.05$); $n = 3$ sessions.

Days of storage	Vitamin C (mg/100 mL)	Total anthocyanins (mg/L)	Total flavanones (mg/L)	Total phenolics (mg/L)	ORAC units (μM trolox equiv./100 mL)
0	61.17 a	69.32 a	106.86 a	1105.82 a	3032.63 a
5	60.84 a	70.39 a	105.97 a	1074.09 a	3092.44 a
10	60.78 a	70.29 a	105.68 a	1041.35 a	3333.79 a
15	61.10 a	70.55 a	98.79 b	899.77 b	2992.62 b
20	59.08 a	70.03 a	95.63 c	813.16 c	2638.24 c
25	51.14 b	58.82 b	95.98 c	813.26 c	2317.78 c
30	51.42 b	58.90 b	94.78 c	813.32 c	2324.06 c

Table 4
Change of sensory attributes in TJ_{CO2} (treatment 3) during refrigerated storage at 4 °C (30 days). Means signed by different letters are significantly different ($p \leq 0.01$); $n = 3$ sessions.

Days of storage	Freshness	Flavour	Acidity	Bitterness	Sweetness	Off-flavour	Colour	Intensity of taste	Intensity of scent
0	5.68 A	5.41 A	5.14 A	3.18 BC	5.05 A	2.86 B	6.95	6.64 A	5.50 A
5	5.82 A	5.05 A	4.50 AB	3.09 C	4.77 A	2.95 B	7.55	6.14 A	5.68 A
10	5.59 A	5.36 A	4.09 B	3.41 BC	4.55 A	2.82 B	7.09	5.91 A	5.68 A
15	5.64 A	5.64 A	3.95 B	3.64 BC	4.14 AB	2.91 B	7.14	6.00 A	5.14 A
20	4.59 AB	5.18 A	4.36 AB	4.32 ABC	2.82 B	3.45 AB	7.32	4.55 B	4.45 A
25	3.91 B	4.64 B	4.59 AB	4.59 AB	2.73 B	5.09 A	7.55	2.41 C	2.77 B
30	2.27 C	3.00 B	4.18 AB	5.36 A	2.68 B	5.45 A	7.32	2.41 C	2.50 B

10, 15, 20, 25, and 30 days of refrigerated storage. For most of the attributes we were interested in, a decreasing trend became evident after 25 days. In fact, the freshness, flavour, intensity of taste and intensity of scent decreased, while off-flavour increased significantly ($p \leq 0.01$) after 25 days, showing a sensory decay of TJ_{CO2} during the last stages of storage. Sensory perception of acidity remained almost constant during storage, with a slight decrease between 10 and 15 days of storage. The stability of the acid medium was probably beneficial to the persistence of colour perception, as the colour of blood orange juice is closely related to the acid pH of the juice (Rapisarda et al., 2000). The bitterness intensity was perceived as being constant during refrigerated storage, but a significant increase ($p \leq 0.01$) of this negative sensory attribute was recorded after 25 days. The sweetness of TJ_{CO2} recorded a significant drop ($p \leq 0.01$) after 20 days, and this decline may be in part due to the use of sugars for undesirable fermentations (Echeverria & Valich, 1989). These results indicate that the juice began to spoil during storage after 20–25 days, even if it does not produce an unacceptable product, as seen before in the microbiological stability study. Hence, we can conclude that HPCD-treated blood orange juice processed at 130 bar, 36 ± 1 °C, 0.385 g_{CO2}/g_{juice} is best before 20 days of refrigerated storage.

4. Conclusions

The results of the present study showed that treatment of blood orange juice with HPCD at lower operative pressure (130 bar) and amount of carbon dioxide (0.385 g_{CO2}/g_{juice}), in line with cost reduction for future operation on an industrial scale, can ensure very good microbial inactivation, extending the shelf-life of fresh orange juice. The nutritional and antioxidant properties of HPCD-treated blood orange juice remained almost unchanged until the 20th day of refrigerated storage, then showing a slight decline, not relevant with respect to the antioxidant protection of the produce. Moreover, the sensory evaluation of the product by a trained panel suggested that HPCD-treated blood orange juice would be well accepted by consumers and, further, that it should be considered best before 20 days of refrigerated storage at 4 ± 1 °C. In fact, our results demonstrate that HPCD treatment cannot be considered as an alternative to traditional thermal methods but as a new milder technology to obtain a stabilised blood orange juice that retains its physicochemical, antioxidant, and sensory properties. This suggests the great potential of HPCD-treated blood orange juice as a new product to be allocated in fresh fruit juice market within a new retail framework, that of freshly squeezed juices with a 20-day shelf-life.

References

Ahrne, L. M., Manso, M. C., Shah, E., Oliveira, F. A. R., & Oste, R. E. (1996). Shelf-life prediction of aseptically packaged orange juice. In T. C. Lee, & H. J. Kim (Eds.), *Chemical markers for processed and stored foods* (pp. 107–117). Washington, DC: American Chemical Society.

Baker, R. A., & Cameron, R. G. (1999). Clouds of citrus juices and juice drinks. *Food Technology*, *53*, 64–69.

Ballestra, P., Abreu Da Silva, A., & Cuq, J. L. (1996). Inactivation of *Escherichia coli* by carbon dioxide under pressure. *Journal of Food Science*, *61*(4), 829–831.

Balaban, M. O., Arreola, A. G., Marshall, M., Peplow, A., Wei, C. I., & Cornell, J. (1991). Inactivation of pectinesterase in orange juice by supercritical carbon dioxide. *Journal of Food Science*, *56*(3), 743–750.

Boff, J. M., Truong, T. T., Min, D. B., & Shellhammer, T. H. (2003). Effect of thermal processing and carbon dioxide-assisted high pressure processing on pectinmethylesterase and chemical changes in orange juice. *Journal of Food Science*, *68*, 1179–1184.

Butz, P., & Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International*, *35*(2–30), 279–284.

Corrales, M., Butz, P., & Tauscher, B. (2008). Anthocyanin condensation reactions under high hydrostatic pressure. *Food Chemistry*, *110*, 627–635.

Del Pozo-Insfran, D., Balaban, M. O., & Talcott, S. T. (2006). Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO_2 processed muscadine grape juice. *Journal of Agricultural and Food Chemistry*, *54*, 5468–5473.

Devlieghere, F., Vermeiren, L., & Debevere, J. (2004). New preservation technologies: Possibilities and limitations. *International Dairy Journal*, *14*, 273–285.

Dillow, A. K., Denghani, F., Hrkack, J. S., Foster, N. R., & Langer, R. (1999). Bacterial inactivation by using near- and supercritical carbon dioxide. *PNAS*, *96*, 10344.

Echeverria, E., & Valich, J. (1989). Enzymes of sugar and acid metabolism in stored Valencia oranges. *Journal of American Society for Horticultural Science*, *114*, 445–449.

Enomoto, A., Nakamura, K., Nagai, K., Hashimoto, T., & Hakoda, M. (1997). Inactivation of food microorganisms by high-pressure carbon dioxide treatment with or without explosive decompression. *Bioscience Biotechnology and Biochemistry*, *61*, 1133–1137.

Garcia-Gonzalez, L., Geeraerd, A. H., Spilimbergo, S., Elst, K., Van Ginneken, L., Debevere, J., et al. (2007). High pressure carbondioxide inactivation of microorganisms in foods: The past, the present and the future. *International Journal of Food Microbiology*, *117*, 1–28.

Garzon, G. A., Wrolstad, R. E., & Durst, R. W. (2002). Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate. *Journal of Food Science*, *67*, 1288–1299.

Gerlach, D., Alleborn, N., Baars, A., Delgado, A., Moritz, J., & Knorr, D. (2008). Numerical simulations of pulsed electric fields for food preservation: A review. *Innovative Food Science and Emerging Technologies*, *9*, 408–417.

Goodner, J. K., Braddock, R. J., & Parish, M. E. (1998). Inactivation of pectinesterase in orange and grapefruit juices by high pressure. *Journal of Agricultural and Food Chemistry*, *46*, 1997−2000.
Ishikawa, H., Shimoda, M., Shiratsuchi, H., & Osajima, Y. (1995). Sterilization of microorganisms by the supercritical CO_2 micro-bubble method. *Bioscience Biotechnology and Biochemistry*, *59*, 1949−1950.
ISO 8586-1 (1993). Sensory analysis — General guidance for the selection, training and monitoring of assessors — Part 1: Selected assessors.
ISO 13299 (2003) Sensory analysis — Methodology — General guidance for establishing a sensory profile.
Hrazdina, G., Borzell, A. J., & Robinson, W. B. (1970). Studies on the stability of the anthocyanin-3, 5-diglucosides. *American Journal of Enology and Viticulture*, *21*, 201.
Jeyamkondan, S., Jayas, D. S., & Holley, R. A. (1999). Pulsed electric field processing of foods: A review. *Journal of Food Protection*, *62*, 1088−1096.
Kimbal, D. (1999). *Citrus processing. Quality control and technology*,New York: AVI Books.
Kincal, D., Hill, W. S., Balaban, M. O., Marshall, M. R., & Wei, C. I. (2005). A continuous high pressure CO_2 system for microbial reduction in orange juice. *Journal of Food Science*, *70*(5), M249−M254.
Kincal, D., Hill, W. S., Balaban, M. O., Portier, K. M., Sims, C. A., & Wei, C. I. (2006). A continuous high-pressure carbon dioxide system for cloud and quality retention in orange juice. *Journal of Food Science*, *71*, C338−C344.
Marcotte, M., Stewart, B., & Fustier, P. (1998). Abused thermal treatment impact on degradation products of chilled pasteurized orange juice. *Journal of Agricultural and Food Chemistry*, *46*, 1991−1996.
Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K., & Hakoda, M. (1994). Disruption of microbial cells by flash discharge of high-pressure carbon dioxide. *Bioscience Biotechnology and Biochemistry*, *58*, 1297.
Ou, B., Hampsch-Woodill, M., & Prior, R. (2001). Developing and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, *49*, 4619−4626.
Parton, T., Bertucco, A., Elvassore, N., & Grimolizzi, L. (2007). A continuous plant for food preservation by high pressure CO_2. *Journal of Food Engineering*, *79*, 1410−1417.
Prior, R.L., Joseph, J.A., Cao, G.,Shukitt-Hale, B. (1999). Can foods forestall aging? *Agricultural Research*, February 1999.
Rapisarda, P., & Intelisano, S. (1996). Sample preparation for vitamin C analysis of pigmented orange juice. *Italian Journal of Food Science*, *8*, 251−256.
Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A., & Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry*, *47*, 4718−4723.
Rapisarda, P., Fanella, F., & Maccarone, E. (2000). Reliability of analytical method for determining anthocyanins in blood orange juice. *Journal of Agricultural and Food Chemistry*, *48*, 2249−2252.
Rapisarda, P., Bellomo, S. E., & Intelisano, S. (2001). Storage temperature effects on blood orange fruit quality. *Journal of Agricultural and Food Chemistry*, *49*, 3230−3235.
Riso, P., Visioli, F., Gardana, C., Grande, S., Brusamolino, A., Galvano, F., et al. (2005). Effects of blood orange juice intake on antioxidant bioavailability and on different markers related to oxidative stress. *Journal of Agricultural and Food Chemistry*, *53*, 941−947.
Rouse, A. H., & Atkins, C. D. (1955). Pectinesterase and pectin in commercial citrus juices as determined by methods used at the citrus experimental station. *Agricultural Experiment Station Journal Series, No. 1141.* (pp. 271−275): University of Florida.
Rouseff, R. L., Martin, S. F., & Yotsey, C. O. (1987). Quantitative survey of narirutin, naringin, hesperidin and neohesperidin in citrus. *Journal of Agricultural and Food Chemistry*, *35*, 1027−1030.
Shomer, R., Cogan, U., & Mannheim, C. H. (1994). Thermal death parameters of orange juice and effect of minimal heat treatment and carbon dioxide on shelf-life. *Journal of Food Processing and Preservation*, *18*, 305−315.
Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin–Ciocalteu reagent. *Methods in Enzimology*, *299*, 152−178.
Spilimbergo, S., Elvassore, N., & Bertucco, A. (2003). Inactivation of microorganisms by supercritical CO_2 in a semi-continuous process. *Italian Journal of Food Science*, *15*, 115−124.

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Variabilità dei componenti salutistici nelle arance bionde e pigmentate

P. Rapisarda - M. Amenta - S. Fabroni - C. Licciardello - D. Pietro Paolo - S. Recupero - G. Rizza - V. Rizzo - M.P. Russo - G. Russo - G. Reforgiato Recupero

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Nell'ultimo trentennio il panorama varietale siciliano delle arance pigmentate (*Citrus sinensis* L. Osbeck) ha subito un profondo mutamento. Si è assistito a una maggiore diffusione della cv. Tarocco (Reforgiato Recupero, 2002) rispetto a Moro e Sanguinello, per cui oggi la varietà Tarocco è nettamente dominante con una produzione a stima nell'ultimo quadriennio di 650.000 t, a cui seguono le varietà Moro con 140.000 t e Sanguinello con quasi 80.000. La preferenza di Tarocco nei nuovi impianti viene certamente attribuita alle sue pregevoli caratteristiche, tanto che Casella (1935) lo definiva "aristocratico".

Oggi l'isolamento di un elevato numero di cloni di Tarocco, contraddistinti da una notevole variabilità per pigmentazione, forma del frutto ed epoca di maturazione, ha ampliato la disponibilità di prodotto da dicembre a maggio. Nell'ambito delle selezioni di Tarocco più richieste vanno menzionati i cloni 57/1E/1, Gallo e Sciara per la dimensione del frutto; Rosso, Lempso, Ippolito, Tapi e TDV per l'elevata pigmentazione; Scirè per il forte attacco del peduncolo e la grana fine del frutto; S. Alfio, Meli e Messina per la tardività.

Va tenuto presente che la diffusione del Tarocco e la scelta di privilegiare la pezzatura del frutto ha determinato la propagazione di cloni con polpa poco pigmentata e non è raro trovare nei mercati, in alcuni periodi dell'anno, frutti non pigmentati. La pigmentazione dei frutti, di cui sono responsabili le antocianine, è determinata da fattori genetici e climatici. Infatti, allo stesso stadio di maturazione la cv Moro presenta sempre il livello di antocianine più elevato rispetto a quella di Tarocco e Sanguinello. Negli ultimi anni sono stati selezionati cloni di Tarocco fortemente pigmentati con concentrazioni di antocianine che si avvicinano a quelli del Moro. Anche il portinnesto gioca un ruolo importante: le varietà innestate su arancio trifogliato producono frutti maggiormente pigmentati rispetto a quelle innestate su arancio amaro o su Citrange Troyer. Infine, un idoneo accumulo di "unità di freddo" nel corso della maturazione dei frutti risulta indispensabile per la biosintesi delle antocianine.

Antocianine e valore nutraceutico delle arance

I frutti di arancio dolce contengono zuccheri (saccarosio, fruttosio e glucosio), acidi organici (principalmente citrico, ma anche malico e isocitrico), carotenoidi (xantofille e caroteni), vitamine (C, A, B1, B6 e B3), polifenoli (flavonoidi ed acidi idrossicinnamici), composti aromatici (esteri, alcoli, aldeidi). Nel succo delle arance rosse si riscontrano concentrazioni più elevate di acidi idrossicinnamici (ferulico, cumarico, caffeico e sinapico), flavanoni (esperidina, narirutina e diosmina) e vitamina C rispetto a quelle contenute nelle varietà a polpa bionda (Rapisarda *et al.*, 1998). Inoltre, esclusivamente nei frutti delle varietà pigmentate sono presenti le antocianine, che si originano dai flavanoni contenuti nell'albedo e nelle membrane attraverso una reazione di riduzione catalizzata da particolari enzimi (Hrazdina, 1982) e si accumulano nelle vescicole acquose dell'endocarpo, essendo solubili in acqua. Nel succo delle cultivar pigmentate sono state identificate circa dieci antocianine fra cui cianidina 3-glucoside e cianidina 3-(6"-malonil) glucoside presenti in maggiore concentrazione (Maccarone *et al.*, 1998). Altre antocianine, presenti in tracce, risultano legate ad altri acidi organici.

Le antocianine determinano particolari qualità attrattive del frutto per il loro colore rosso brillante, ma esercitano un ruolo ancora più importante per le loro proprietà farmacologiche e antiossidanti. Le antocianine possono ridurre lo stress ossidativo dell'organismo umano, promuovendo l'attivazione degli enzimi di detossificazione specifici. È infatti ormai dimostrato che l'eccessiva produzione di radicali liberi può abbassare la capacità antiossidante, a livello cellulare, fornita dagli enzimi quali glutatione perossidasi, catalasi e superossido dismutasi, e dai composti antiossidanti endogeni come il glutatione (Dröge, 2002; Fang *et al.* 2002). Da questa prospettiva diventa evidente che gli aspetti benefici delle antocianine nei meccanismi di difesa sono di particolare interesse.

Le antocianine sono state associate a potenziali effetti benefici nei confronti di diverse patologie quali la fragilità capillare, la retinopatia diabetica e l'aterosclerosi (Mazza, 2000; Rapisarda *et al.*, 2001; Crozier *et al.*, 2009; de Pascual-Teresa *et al.*, 2010). Inoltre, gli aspetti farmacologici delle antocianine del succo delle arance rosse sono stati studiati in modelli *in vitro* e *in vivo*. In particolare, Saija *et al.* (1992) ha dimostrato che l'assunzione di succo di Moro, la varietà di arancio più ricca di antocianine, può indurre effetti protettivi sulla mucosa gastrica e sembra produrre anche un effetto immunostimolante. In un recente studio, inoltre, è stato provato che la somministrazione di succo di Moro riduce in maniera significativa il peso corporeo e contrasta l'accumulo di grassi in topi sottoposti a dieta ipercalorica (Titta *et al.*, 2009).

L'elevato potere antiossidante delle antocianine e dei polifenoli in genere ha dato origine ad un nuovo segmento di mercato definito "superfruits", cioè

TAB. 1 - CONTENUTO DEI PARAMETRI SALUTISTICI IN DIVERSE CULTIVAR DI ARANCIO E NEL GERMOPLASMA PIGMENTATO E BIONDO (CAMPIONAMENTO FEBBRAIO 2008)

Tipologia di arance	Acido Ascorbico (mg/100mL)	Unità ORAC (µmolTE/100m)	Polifenoli totali (mg/L)	Antocianine totali (mg/L)
a) Pigmentate				
Tarocco	61,4 A	1734,5 C	771,2 BC	16,7 C
Moro	46,2 BC	2839,0 B	817,8 BC	127,3 B
Sanguinello e Doppio Sanguigno	61,5 A	1973,3 C	912,0 B	18,9 C
Ibridi pigmentati di mandarino	32,2 C	6726,1 A	1504,2 A	545,5 A
b) Bionde				
Navel	51,7 B	1494,6 C	775,5 BC	-
Valencia	57,8 AB	1384,7 C	739,9 BC	-
Vaniglia	29,5 C	1117,8 C	564,8 C	-
*Germoplasma pigmentato**	*59, 4 A*	*2033,9 A*	*823,0 a*	*44,8*
*Germoplasma biondo***	*50,5 B*	*1428,3 B*	*744,1 b*	-

- Lettere maiuscole p≤0,01; Lettere minuscole p≤0,05 secondo il test di Tukey.
* valori medi del gruppo delle arance pigmentate.
** valori medi del gruppo delle arance bionde

TAB. 2 - COEFFICIENTE DI CORRELAZIONE (R) TRA COMPONENTI FUNZIONALI NELL'AMBITO DEL GERMOPLASMA DI ARANCE PIGMENTATE IN VARI AMBIENTI DI COLTIVAZIONE

	Acido Ascorbico (mg/100mL)	Polifenoli (mg/L)	Antocianine (mg/L)
ORAC	-0,37	0,73*	0,91*
Acido ascorbico	-	-0,10	-0,54
Polifenoli	-	0,70*	-

Gradi di libertà 148; * p≤ 0,01

TAB. 3 - COEFFICIENTE DI CORRELAZIONE (R) TRA COMPONENTI FUNZIONALI NELL'AMBITO DEL GERMOPLASMA DI ARANCE BIONDE NEI VARI AMBIENTI DI COLTIVAZIONE

	Acido ascorbico (mg/L)	Polifenoli (mg/L)
ORAC	0,082	0,33
Acido ascorbico	-	0,66*

Gradi di libertà 25; * P≤ 0,01

frutti pigmentati che hanno un contenuto particolarmente alto di sostanze antiossidanti e che ne fanno quindi un vero e proprio "alimento funzionale". Per quantificare il potere antiossidante degli alimenti recentemente è stato sviluppato un metodo chiamato ORAC ("Oxygen Radicals Absorbance Capacity"), idoneo a valutare la capacità di assorbimento dei radicali liberi prodotti dall'ossigeno da parte di sistemi complessi quali sono gli alimenti. In definitiva, il test ORAC può dare una stima della capacità di "scavenging" ("spazzare via") degli alimenti contro i radicali perossidici ($ROO^{\cdot}$) e idrossilici ($OH^{\cdot}$) prodotti dal nostro organismo nel corso del normale metabolismo cellulare o quando sottoposto a stress.

Al fine di migliorare le conoscenze sulle proprietà funzionali delle arance pigmentate è stata effettuata la presente ricerca con lo scopo di:

- individuare, nell'ambito del vasto patrimonio genetico di arance e ibridi pigmentati, i valori dei principali parametri responsabili delle funzioni salutistiche del frutto e le loro correlazioni;
- verificare, in varietà di arancio medio-precoci e tardive, attraverso campionamenti di frutti effettuati in epoche progressive, l'influenza della maturazione sui principali parametri responsabili del valore salutistico e su alcuni geni strutturali implicati nella biosintesi delle antocianine.

Materiali e metodi

Campionamento

Per la correlazione dei parametri responsabili del valore salutistico il campionamento, effettuato la prima settimana di febbraio 2008, ha riguardato un ampio germoplasma proveniente da 5 aree pedoclimatiche così dislocate: 2 in Sicilia, 2 in Calabria, 1 in Basilicata. Ogni campione di dieci frutti era replicato 3 volte. Il campionamento ha riguardato complessivamente 59 situazioni diversificate per genotipo o per area, di cui 38 sono riconducibili a selezioni di Tarocco, 3 a Moro, 7 a Sanguinello e Doppio Sanguigno o altre accessioni pigmentate, 2 a ibridi di mandarino pigmentato (OTA 9 = clementine Oroval × arancio Tarocco e Amoa 8 = arancio Moro × mandarino Avana), 9 a cultivar bionde.

Per valutare l'influenza della maturazione sui parametri funzionali e sui livelli di espressione di calcone sintasi (CHS), antocianidina sintasi (ANS), UDP-glucosio, flavonoide-3-O-glucosiltransferasi (UFGT) sono state prese in considerazione 5 cultivar pigmentate medio-precoci e 2 tardive. In entrambi i casi è stato incluso anche un genotipo biondo come controllo. Il campionamento di ogni cultivar, effettuato nel 2007 nell'azienda sperimentale del CRA-ACM a Palazzelli, ha riguardato tre progressivi stadi di maturazione: rispettivamente i primi di gennaio (I prelievo), febbraio (II prelievo) e marzo (III prelievo) per i genotipi medio-precoci [OTA 9, Moro, Tarocco Sciara, Gallo, Rosso, Washington Navel]; aprile (I prelievo), maggio (II prelievo) e giugno (III prelievo) per quelli tardivi (Tarocco Messina, Ovale, Valencia). Ogni campione è stato replicato 3 volte, con dieci frutti per replicazione.

Analisi

I parametri fisico-chimici, quali acidità totale (AT) e solidi solubili totali (SST), sono stati determinati secondo metodi standard (Kimbal, 1999). La determinazione del contenuto di acido ascorbico è stata effettuata mediante HPLC (Rapisarda *et al.*, 1996). Le antocianine, espresse come mg/l di cianidina-3-glucoside, sono state valutate spettrofotometricamente (Varian UV-Vis spectrophotometer mod. Cary 100 Scan) secondo il metodo del pH differenziale (Rapisarda *et al.*, 2000). I polifenoli totali sono stati determinati con il metodo spettrofotometrico di Folin-Ciocalteu (Singleton *et al.*, 1999). L'attività antiossidante è stata valutata mediante test ORAC, come descritto da Cao *et al.* (1999) con opportune modifiche. I risultati sono stati riportati come micromoli di Trolox equivalenti (TE) per 100 ml di succo.

La metodologia utilizzata per l'estrazione di RNA e per la Real Time-PCR quantitativa è stata precedente-

TAB. 4 - EFFETTI DELLO STADIO DI MATURAZIONE SULLE PROPRIETÀ SALUTISTICHE DI DIVERSI GENOTIPI DI FRUTTI DI ARANCIO

Varietà	Prelievo[1]	SST/AT[2]		Antocianine totali (mg/l)		Acido ascorbico (mg/100 ml)		Polifenoli totali (mg/L)		Unità ORAC[3]		Analisi molecolari CHS[4]	ANS[5]	UFGT[6]
Cultivar medio-precoci														
a) Pigmentate														
TAROCCO ROSSO	I	7,5	B	38,7	b	65,9	B	926,3	B	1513,5	C	2280	116	260
TAROCCO ROSSO	II	9,3	AB	40,5	b	71,7	AB	986,2	B	3043,9	B	1876	123	148
TAROCCO ROSSO	III	11,9	A	66,8	a	77,6	A	1737,0	A	3763,7	A	6348	521	222
MORO NUCELLARE	I	5,9	B	60,6	C	47,9	B	795,4	C	1894,4	C	7407	586	389
MORO NUCELLARE	II	7,5	AB	150,5	B	51,9	AB	1090,2	B	3446,1	B	5029	305	322
MORO NUCELLARE	III	8,5	A	212,7	A	59,9	A	1482,3	A	4898,9	A	11281	958	620
TAROCCO GALLO	I	6,9	B	1,4	b	62,3		772,0		1590,3	B	1709	128	66
TAROCCO GALLO	II	8,6	B	18,0	ab	61,2		731,4		2237,5	A	1361	110	160
TAROCCO GALLO	III	11,3	A	23,6	a	61,4		752,5		2342,9	A	3852	443	252
TAROCCO SCIARA	I	6,1	C	3,5	B	64,5		667,1	b	1881,0		2404	132	137
TAROCCO SCIARA	II	8,0	B	4,4	B	67,4		687,3	ab	1939,6		1463	60	95
TAROCCO SCIARA	III	10,1	A	13,4	A	81,6		758,4	a	1993,5		4272	433	318
OTA 9	I	6,3	B	458,0	B	68,1		1791,5	b	4521,9	B	8937	794	1153
OTA 9	II	8,2	AB	814,2	A	64,4		2933,0	a	10284,4	A	2085	115	472
OTA 9	III	9,1	A	680,2	A	68,6		2635,3	ab	10205,6	A	5617	941	975
b) Bionde														
WASHINGTON NAVEL	I	11,3	B			61,3		620,3	b	1613,3	B	13	0	0
WASHINGTON NAVEL	II	15,6	A			63,2		636,1	b	2117,0	A	5,1	0	0
WASHINGTON NAVEL	III	18,0	A			65,5		746,5	a	2099,6	A	4,8	0	0
Cultivar tardive														
a) Pigmentate														
TAROCCO MESSINA	I	10,2		1,8		50,5		609,0		1698,4	A	145	1	1
TAROCCO MESSINA	II	10,7		1,5		50,8		689,0		1473,1	B	412	35,4	12
TAROCCO MESSINA	III	11,3		1,7		49,5		645,6		1512,4	AB	295	6,2	10
b) Bionde														
OVALE	I	9,87	B			62,0	A	756,8		1955,8	A	3,3	0	0
OVALE	II	13,74	A			48,0	B	727,3		1474,5	B	6,1	0	0
OVALE	III	14,01	A			48,6	B	724,1		1440,3	B	11,8	0	0
VALENCIA	I	9,66				54,4		860,7		1927,0	a	2,0	0	0
VALENCIA	II	11,34				56,3		774,4		1568,1	ab	7,5	0	0
VALENCIA	III	11,18				52,3		679,7		1439,9	b	1	0	0

Significatività: lettere minuscole, $p \leq 0,05$; lettere maiuscole, $p \leq 0,01$; assenza di lettera, ns.
[1]Campionamenti effettuati nel 2007: i primi di gennaio (I prelievo), febbraio (II prelievo) e marzo (III prelievo) per i genotipi medio-precoci; aprile (I prelievo), maggio (II prelievo) e giugno (III prelievo) per quelli tardivi.
[2]Solidi Solubili Totali/Acidità Totale; [3]Oxygen Radicals Absorbance Capacity; [4]Calcone Sintasi; [5]Antocianidina Sintasi; [6]UDP-Glucosio: Flavonoide-3-O-Glucositransferasi.
Livello di espressione indicato in "mRNA fold increase".

mente riportata in Licciardello *et al.* (2008).

L'elaborazione statistica dei risultati è stata effettuata utilizzando il software MSTAT WIN 10. È stata effettuata l'analisi della varianza (Anova) e le medie sono state separate con il test di Tukey.

Risultati e discussione

La tabella 1 riporta i valori medi dei parametri responsabili del valore salutistico delle varietà pigmentate e di quelle bionde provenienti da tre regioni dell'Italia meridionale (Sicilia, Calabria e Basilicata). Nelle cultivar pigmentate l'acido ascorbico ha evidenziato valori più elevati in Tarocco e nel gruppo Sanguinello e Doppio Sanguigno rispetto a Moro. Gli ibridi pigmentati evidenziano i valori più bassi probabilmente per la loro discendenza dal mandarino.

Nell'ambito del gruppo pigmentato è stato riscontrato il livello più elevato di ORAC negli ibridi pigmentati, seguiti dal Moro, mentre il Tarocco, che tendenzialmente mostra livelli più elevati del biondo, non ne risulta statisticamente affetto. Pur considerando l'eterogeneità del campionamento effettuato, è emerso un più elevato contenuto in unità ORAC nel germoplasma pigmentato rispetto al biondo, determinato principalmente dalla presenza delle antocianine. Dall'analisi mostrata nella tabella 2 si osserva, all'interno del germoplasma pigmentato, una correlazione positiva fra

ORAC, polifenoli e antocianine, a differenza della vitamina C, che è risultata non significativa. I nostri dati concordano con quelli precedentemente riportati da Rapisarda *et al.* (1999) in cui veniva evidenziato un peso minore della vitamina C sul potere antiossidante rispetto ai polifenoli totali. Nelle cultivar bionde (Tab. 3), invece, si osserva una correlazione positiva esistente tra contenuto ORAC e vitamina C, probabilmente per la mancanza del ruolo esercitato dalle antocianine. I meccanismi che controllano il contenuto di vitamina C nei frutti sono ancora scarsamente compresi, ma il suo livello può essere attribuibile ad un bilanciamento di biosintesi, ossidazione e ricircolo ("recycling"). Recentemente (Yang *et al.*, 2011) sono state evidenziate differenze nei contenuti di vitamina C e nei geni coinvolti nella biosintesi tra la polpa di arancio dolce e di satsuma.

I risultati complessivi inerenti le analisi effettuate sulle accessioni a confronto, separate per ciascun prelievo, sono riportati nella tabella 4. È evidente l'andamento crescente dell'indice di maturazione SST/AT in tutti i genotipi, dovuto principalmente ad un decremento dell'acidità totale legato al più avanzato stadio di maturazione. È infatti ampiamente riportato che la concentrazione di acido citrico diminuisce progressivamente, poiché con gli altri acidi organici fornisce scheletri carboniosi per la sintesi di composti fenolici (Kalt *et al.*, 1999). Infatti, il contenuto in antocianine totali nei cloni di Tarocco Sciara, Gallo e Rosso e in Moro è aumentato con il progredire della maturazione, raggiungendo al terzo prelievo concentrazioni più elevate, a eccezione dell'ibrido OTA 9, che invece ha evidenziato il più alto contenuto (~ 815 ppm) nel secondo prelievo, e di Tarocco Messina, il cui contenuto non risulta variare nei diversi campionamenti. Anche il livello di polifenoli totali è aumentato nel corso della maturazione per tutti i genotipi a maturazione medio-precoce, ad eccezione di Tarocco Gallo, mentre per le cultivar tardive la concentrazione di polifenoli totali è rimasta costante nei diversi campionamenti. Il livello di acido ascorbico, infine, con il progredire della maturazione è aumentato significativamente nei genotipi Tarocco Rosso e Moro, mentre è diminuito nella varietà tardiva Ovale. Circa l'indice che misura l'attività antiossidante del

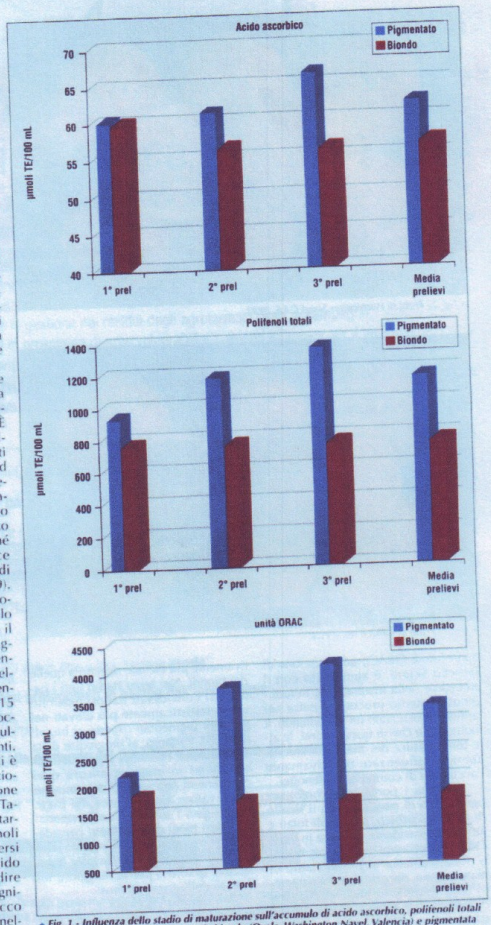
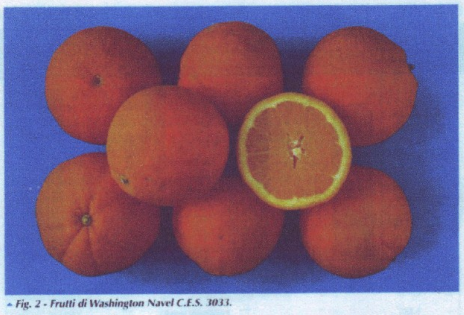


Fig. 1 - Influenza dello stadio di maturazione sull'accumulo di acido ascorbico, polifenoli totali ed unità ORAC nei genotipi di arancia bionda (Ovale, Washington Navel, Valencia) e pigmentata (Tarocco Rosso, Gallo, Sciara, Messina, Moro nucellare, OTA 9).



▲ *Fig. 2 - Frutti di Washington Navel C.E.S. 3033.*

▲ *Fig. 3 - Frutti di Tarocco rosso VCR.*

frutto (unità ORAC), ad eccezione di Tarocco Sciara, è aumentato con il procedere della maturazione in tutti i genotipi medio-precoci, mentre nei tardivi si è osservato un decremento. È ipotizzabile che in questi ultimi, le alte temperature dei mesi primaverili abbiano influenzato negativamente l'accumulo di composti polifenolici.

La figura 1 pone a confronto, per ogni stadio di maturazione, il livello di acido ascorbico, polifenoli totali e attività antiossidante espressa in unità ORAC nei genotipi biondi e pigmentati. Relativamente al primo stadio di maturazione, per nessuno dei parametri considerati sono state evidenziate differenze significative. Nel secondo stadio le unità ORAC relative ai genotipi pigmentati sono risultate significativamente più elevate rispetto a quelle dei biondi. Nel terzo stadio tutti i parametri considerati hanno mostrato valori statisticamente più elevati nei genotipi pigmentati rispetto ai biondi ($p \leq 0,05$). Pertanto, al progredire della maturazione, i genotipi pigmentati accumulano in maggior misura quei componenti nutraceutici determinanti per il valore antiossidante dei frutti. Nella figura 1 sono stati anche riportati i valori medi dei parametri considerati nei tre prelievi, da cui si evidenziano differenze statisticamente significative ($p \leq 0,01$) per la maggiore influenza dell'ultimo prelievo.

Per quanto riguarda i livelli di espressione, misurati mediante Real Time-PCR, dei geni strutturali CHS, ANS e UFGT implicati rispettivamente a monte (CHS) e a valle (ANS e UFGT) della biosintesi dell'antocianina, i risultati (Tab. 4) evidenziano livelli più alti nelle cultivar medio-precoci dell'indice "mRNA fold increase" rispetto all'unica cultivar pigmentata tardiva caratterizzata anche da un basso contenuto di antocianine. Generalmente, i dati del secondo campionamento presentano valori più bassi rispetto al primo ed al terzo campionamento, non correlabili al contenuto di antocianine dello stesso periodo, perché i dati trascrizionali dell'RNA sono legati all'attività espressa nel momento del prelievo, mentre i contenuti di antocianina esprimono un processo di accumulo. Questi risultati dimostrano che la componente fenolica e il livello di antocianine, in particolare, possono essere considerati come i più importanti fattori che determinano il valore antiossidante in termini di unità ORAC dei succhi pigmentati, suggerendo il consumo di succo d'arancia rossa come prodotto dotato di qualità funzionali superiori a quello delle bionde.

Conclusioni

Il germoplasma pigmentato dell'arancio è caratterizzato da un notevole numero di accessioni, per la maggior parte isolate dalla cv Tarocco (Retorgiato, 2002). Il consumatore, per le mutate esigenze dietetiche e salutistiche, richiede, almeno per alcune nicchie di mercato, frutti in cui il valore salutistico sia alto, costante e certificabile. Su queste istanze si basa il crescente interesse verso le arance pigmentate e sul loro contenuto di vitamina C, antocianine e antiossidanti in genere.

Questa ricerca ha confermato che le arance a polpa rossa si distinguono dalle altre varietà bionde o gialle per il più elevato contenuto di queste sostanze e, nella maggior parte dei genotipi pigmentati, i componenti salutistici aumentano col progredire della maturazione.

RIASSUNTO

Le arance pigmentate, per la presenza di antocianine e il conseguente valore nutraceutico, rappresentano un punto di forza dell'agrumicoltura italiana.

Al fine di migliorare le conoscenze sulle proprietà funzionali delle arance pigmentate è stata effettuata una ricerca con l'obiettivo di: i) individuare, nel confronto fra germoplasma di arance rosse e bionde, le correlazioni esistenti, all'interno di ciascun gruppo, fra i parametri che influenzano il valore salutistico (antociani-

▲ *Fig. 4 - L'ibrido OTA 9 deriva da incrocio di clementine Oroval e arancio Tarocco.*

▲ *Fig. 5 - Frutti di Moro 58-8D-1.*

ne, acido ascorbico, polifenoli, ORAC); ibi valutare gli stessi parametri e i livelli di espressione di alcuni geni strutturali implicati nella biosintesi delle antocianine, durante la maturazione di alcune cultivar medio-precoci e tardive. I risultati ottenuti hanno evidenziato che i frutti del germoplasma pigmentato mostravano un più alto livello di sostanze antiossidanti rispetto a quelli del biondo. Inoltre nelle cultivar pigmentate medio-precoci è stato osservato un incremento degli indici salutistici al progredire della maturazione.

SUMMARY

The blood oranges, thanks to their anthocyanins content and nutraceutical value, represent a crucial production of the Italian citriculture.

The present research has been addressed to: a) identify the mean values and the correlations among the parameters affecting the antioxidant values (anthocyanins, vitamin C, polyphenols, ORAC) in blood and common germplasm; b) evaluate the same parameters and the expression levels of the structural genes involved in the biosynthesis of anthocyanins during ripening of some medium-early and late cultivars. The results showed that fruits belonging to a blood germplasm had higher levels of antioxidants than the common ones. In addition in medium-early blood cultivars antioxidant parameters increased during ripening.

BIBLIOGRAFIA

Cao G., Prior R. L. (1999) - Measurement of Oxigen Absorbance Capacity in Biological Samples. Methods in Enzymology, 299, 50-62.

Casella D. (1935) - L'Agrumicoltura siciliana. Annali della R. Stazione Sperimentale di Agrumicoltura e Frutticoltura, 1-149.

Crozier A., Jaganath I. B., Clifford M. N. (2009) - Dietary phenolics: chemistry, bioavailability and effects on health. Nat. Product Rep., 26, 1001-1043.

De Pascual-Teresa S., Moreno D. A., Garcia-Viguera C. (2010) - Flavanols and anthocyanins in cardiovascular health. A review of current evidence. Int. J. Mol. Sci, 11, 1679-1703.

Dröge W. (2002) - Free radicals in the physiological control of cell function. Physiological Reviews, 82, 47–95.

Fang Y.-Z., Yang S., Wu G. (2002) - Free radicals, antioxidants, and nutrition. Nutrition, 18, 872-879.

Hrazdina G. (1982) - Anthocyanins. The Flavonoids. Advances in Research. Harborne, Mabry, Ed., Chapman and Hall, London and New York, p.169.

Kalt W., Forney C.F., Martin A., Prior R. (1999) - Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. J. Agric. Food Chem., 47, 4638-4644.

Kimbal D. (1999) - Citrus Processing. Quality Control and Technology. AVI Books, New York.

Licciardello C., Russo M. P., Valè G., Reforgiato Recupero G. (2008) - Identification of differentially expressed genes in the flesh of blood and common oranges. Tree Genetics & Genomes, 4, 315-331.

Maccarone E., Rapisarda P., Fanella F., Arena E., Mondello L. (1998) - Cyanidin-3-(6"-malonyl)- -glucoside, One of the major anthocyanins in blood orange juice. Ital. J. Food Sci., 10, 367-372.

Mazza G. (2000) - Health aspects of natural colours. In G. J. Lauro, & F. J. Francis (Eds.), Natural food colorants. Science and Technology. Basel/New York: Marcel Dekker, 289-314.

Rapisarda P., Intelisano S. (1996) - Sample preparation for vitamin C analysis of pigmented orange juices. Ital. J. Food Science, 3, 251-256.

Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., Maccarone, E. (1998) - Hydroxycinnamic acids as markers of Italian blood orange juices. J. Agric. Food Chem., 46, 464-470.

Rapisarda P, Tomaino A, Lo Cascio R, Bonina F, De Pasquale A, Saija A. (1999) - Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. J Agric Food Chem, 47, 4718-4723.

Rapisarda, P., Fanella, F., Maccarone, E. (2000) - Reliability of analytical method for determining anthocyanins in blood orange juice. J. Agric. Food Chem., 48, 2249-2252.

Rapisarda P., Bellomo S.E., Intrigliolo F. (2001) - Anthocyanins in blood oranges: composition and biological activity. Recent Res. Devel. Agricultural & Food Chemistry, 5, 217-230.

Reforgiato Recupero G. (2002) - La propagazione dell'arancio Tarocco e del clementine Comune. Italus Hortus, Vol. 9 N. 3, 81-84.

Saija, A., Scalese, M., Imbesi, A., Princi, I. and Di Giacomo, A. (1992) - Anthocyanins of Moro orange fruit juice: pharmaceutical aspects. Proc. Int. Soc. Citriculture, 13, 1127.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999) - Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods in Enzimology, 299, 152-178.

Titta L., Trinei M., Stendardo M., Berniakovich I., Petroni K., Tonelli C., Riso P., Porrini M., Minucci S., Pelicci P.G., Rapisarda P., Reforgiato Recupero G., Giorgio M. (2009) Intl. J. of Obesity, 1, 1-11.

Yang X.Y., Xie J.X., Wang F.F., Zhong J., Liu Y.Z., Li G.H., Peng S.A. (2011) - Comparison of ascorbate metabolism in fruits of two citrus species with obvious difference in ascorbate content in pulp. J Plant Physiol., 168, 2196-2205.

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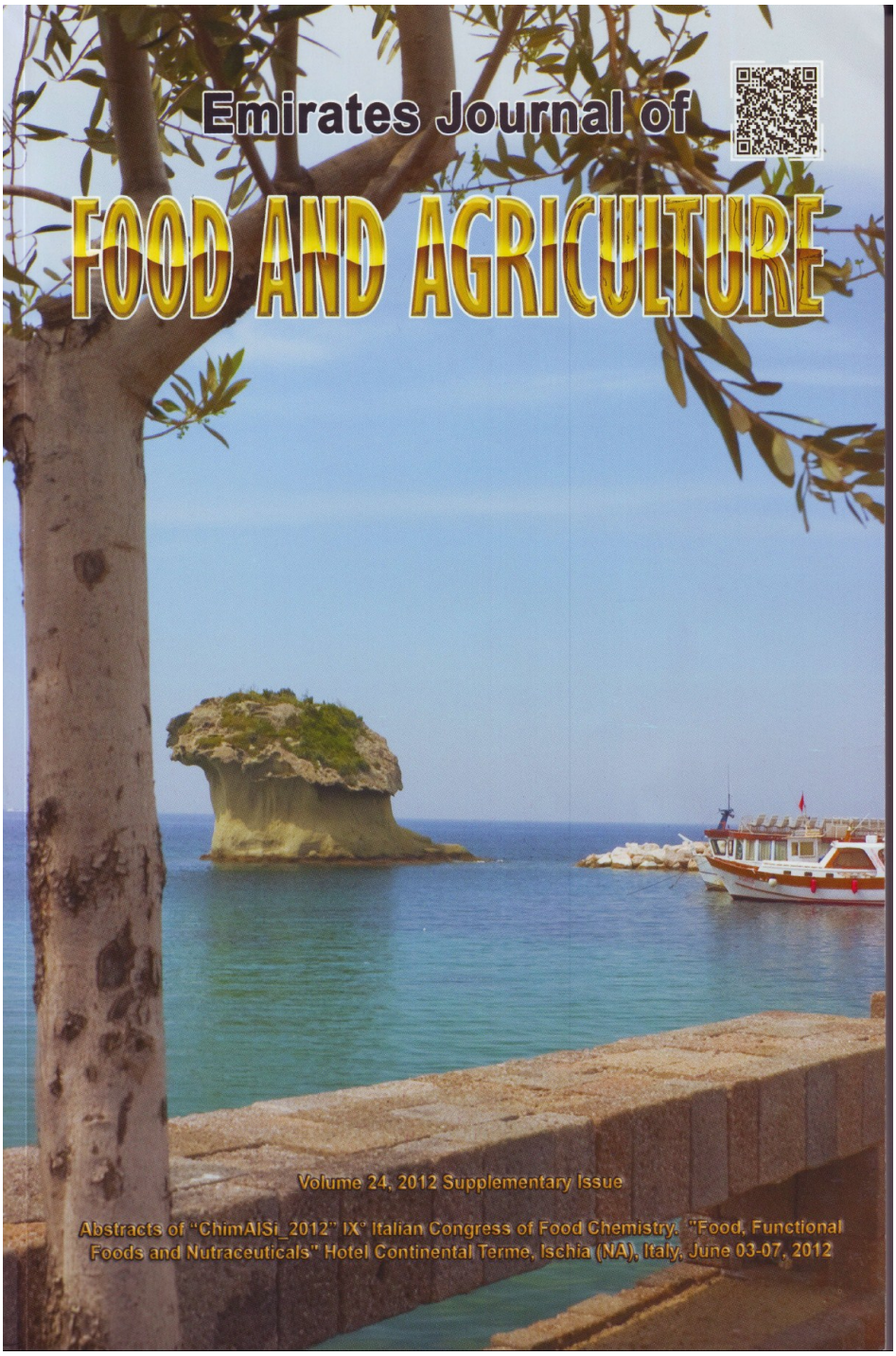
Emirates Journal of
FOOD AND AGRICULTURE
Volume 24, 2012 Supplementary Issue
Abstracts of "ChimAlSi_2012" IX° Italian Congress of Food Chemistry. "Food, Functional Foods and Nutraceuticals" Hotel Continental Terme, Ischia (NA), Italy, June 03-07, 2012

NUOVI AGRUMI CON ELEVATE PROPRIETA' FUNZIONALI

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INTRODUZIONE

I frutti di agrumi sono conosciuti ed apprezzati dai consumatori perché ricchi di vitamina C ed altri composti bioattivi tra cui flavonoidi ed acidi idrossicinnamici. I flavanoni glicosidi (esperidina, narirutina, naringina, neoesperidina) sono i polifenoli più abbondanti nei frutti di agrumi, ma sono presenti anche concentrazioni significative di altri flavonoidi tra cui flavoni metossilati, flavonoli ed antocianine, quest'ultime solo nelle arance pigmentate [1]. Studi precedenti hanno dimostrato che l'esperidina, presenta proprietà antiossidante, anticarcinogenica ed antinfiammatoria [2]. Gli acidi idrossicinnamici possiedono una significativa attività antiossidante ed azione chemoprotettiva, come dimostrato da studi *in vitro* ed *in vivo* [3]. Le antocianine sono state anche associate a potenziali effetti benefici nei confronti di diverse malattie come la fragilità capillare, la retinopatia diabetica e l'aterosclerosi [4]. Recenti ricerche hanno evidenziato che i succhi delle arance pigmentate "Moro", "Tarocco" e "Sanguinello" esercitano un effetto antiossidante superiore rispetto ai succhi di arance bionde e questa attività è direttamente correlata al contenuto di antocianine ed al maggior livello di vitamina C, flavanoni ed acidi idrossicinnamici [5,6].

Il CRA – Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee dal 1973 ha avviato un programma di "breeding" volto al miglioramento genetico delle varietà agrumicole pigmentate esistenti ed allo sviluppo di nuovi ibridi pigmentati, caratterizzati da elevata pezzatura, facile sbucciatura e nuove ed originali caratteristiche organolettiche, per allargare il mercato del consumo fresco dei frutti e dei prodotti trasformati.

Allo scopo di valutare l'ereditarietà di caratteri rilevanti per l'attività biologica, quali l'accumulo di vitamina C, antocianine, flavanoni ed acidi idrossicinnamici, è stato avviato uno studio sulla composizione chimica e la capacità antiossidante del succo di nuovi ibridi pigmentati e non pigmentati appartenenti al gruppo OTA [clementine 'Oroval' (*Citrus clementina* Hort. Ex Tan.) x arancio Tarocco (*Citrus sinensis* L. Osbeck)] e al gruppo OMO [clementine 'Oroval' (*C. clementina* Hort. Ex Tan.) x arancio Moro (*C. sinensis* L. Osbeck)]. Nel succo dell'ibrido OMO 31 e in quello dei suoi genitori è stata, inoltre, valutata l'influenza del periodo di maturazione sull'accumulo dei componenti dotati di attività biologica. Infine, è stato caratterizzato il profilo chimico-fisico, nutrizionale e dei polifenoli del succo dei frutti di due nuovi ibridi triploidi pigmentati, il Tacle e il Clara [clementine Monreal (*C. clementina* Hort. Ex Tan.) x arancio Tarocco (*C. sinensis* L. Osbeck)] ampiamente diffusi in Italia, durante 104 giorni di frigoconservazione a 6±1°C e 90-95% UR, al fine di valutare l'evoluzione di questi componenti durante il trattamento post-raccolta.

MATERIALI E METODI

Campionamenti

I frutti degli ibridi OTA 8, OTA 9, OTA 20, OTA 31, OTA 43, OMO 24 ed OMO 30 e dei rispettivi genitori sono stati raccolti a maturazione da tre piante per ogni genotipo. In particolare, il clementine

'Oroval' è stato raccolto a dicembre, il Tarocco '57–1E-1' a febbraio, mentre il Moro e tutti gli ibridi sono stati raccolti a marzo. I frutti dell'ibrido OMO 31 e dei genitori clementine 'Oroval' e Moro sono stati raccolti, da tre piante per ogni genotipo, a sei differenti stadi di maturazione, nel periodo compreso tra novembre e marzo. I frutti degli ibridi Tacle e Clara sono stati raccolti a febbraio, da tre piante per ogni genotipo. Campioni (100 kg ca.) di ciascun ibrido sono stati frigoconservati a 6±1°C e 90–95% di umidità relativa per 104 giorni e le analisi sono state condotte su 20 frutti prelevati ad intervalli di 20–30 giorni.

Analisi fisico-chimiche e della componente antiossidante

I parametri fisico-chimici dei frutti quali resa succo, pH, acidità totale (AT) e solidi solubili totali (SST) sono stati determinati secondo metodi standard. La determinazione del contenuto di acido ascorbico è stata effettuata mediante HPLC [7]. Le antocianine, espresse come mg/L di cianidina-3-glucoside, sono state analizzate spettrofotometricamente [8]. I polifenoli totali sono stati determinati con il metodo di Folin-Ciocalteu [9]. I flavanoni glucosidi e gli acidi idrossicinnamici sono stati valutati mediante HPLC [10, 11]. I carotenoidi totali sono stati determinati spettrofotometricamente [12].

RISULTATI

La progenie dell'incrocio clementine x arancio, denominata OTA e OMO, ha prodotto ibridi con differenti livelli di zuccheri ed acidi (Tab. 1). I valori dei rapporti SST/AT suggeriscono che gli ibridi OTA 9, OTA 31, OMO 24 ed OMO 30 maturano a marzo, mentre l'OTA 43 raggiunge prima l'epoca di maturazione. Il contenuto di acido ascorbico della maggior parte degli ibridi (Tab. 2) è risultato più in alto rispetto quello del genitore femminile (clementine). Le maggiori concentrazioni sono state rilevate nell'OTA 20 e nell'OTA 31, con valori superiori rispetto all'arancia Tarocco rispettivamente del 16% e del 25%. Il contenuto in polifenoli totali (Tab. 2) è risultato mediamente compreso fra quello dei genitori, con valori più alti negli ibridi maggiormente ricchi in acido ascorbico e flavanoni totali. L'ibrido OTA 9 ha mostrato il valore più elevato in polifenoli totali (1554.67 mg/L), mentre i livelli più bassi sono stati riscontrati nell'ibrido OTA 8 (Tab. 2). Il succo dell'ibrido OTA 9 contiene anche maggiori concentrazioni di flavanoni ed acidi idrossicinnamici rispetto agli altri ibridi ed ai rispettivi genitori (Tab. 3).

Lo studio dell'evoluzione dei componenti biologicamente attivi nel corso della maturazione dei frutti dell'ibrido OMO 31 e dei rispettivi genitori, ha mostrato che il contenuto di acido ascorbico del nuovo ibrido, si è sempre mantenuto intermedio tra quello dei genitori, fino a maturazione (gennaio) quando i valori sono risultati uguali (Fig. 1). La concentrazione di flavanoni glucosidi nel succo dell'ibrido OMO 31 ha messo in evidenza un netto incremento a partire da dicembre, raggiungendo valori superiori a 400 mg/L a febbraio, che risultano nettamente più alti rispetto a quelli riscontrati nel genitore maschile (Moro) (Fig. 2). La concentrazione di antocianine nel succo dell'ibrido OMO 31 si è mantenuta sempre più elevata rispetto a quella dell'arancia Moro (Fig. 3) ed in particolare, a febbraio, è risultata circa il doppio (265.96 mg/L) di quella del genitore maschile (132.42 mg/L). L'evoluzione degli acidi idrossicinnamici totali (Fig. 4) ha mostrato lo stesso "trend" ed in marzo la concentrazione di questi composti nel succo dell'ibrido (121.18 mg/L) è risultata doppia rispetto a quella del Moro (67.52 mg/L).

La frigoconservazione dei frutti degli ibridi Tacle e Clara (Tab. 4) ha causato una riduzione della resa in succo e dell'acidità ed al tempo stesso un leggero incremento dei solidi solubili totali. La

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S21P04

Change in nonvolatile flavours of blood and common orange fruits during cold storage

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Prolonged cold storage may cause relevant changes in volatile and non-volatile components of orange fruit [*Citrus sinensis*], probably associated with the decrement of characteristic fruit flavour and sensory acceptability. The aim of this work was to evaluate the changes of nonvolatile components of two blood varieties ('Tarocco' and 'Moro') and a common variety ('Washington navel') of orange fruits stored at 6 ± 1 °C and 90-95 % RH for 60 days. Taste components such as total and individual sugars (TSS, glucose, fructose and sucrose) and acids (titratable acidity, ascorbic acid) were determined during fruit storage. In addition, other compounds influencing the sensory characteristics of the fruit such as total anthocyanins, putrescine and limonin were evaluated. Sensory assessments were performed by a trained sensory panel at 15 days intervals. TSS and TA did not show significant variations during storage in all varieties. Such a trend was confirmed by evaluating glucose, fructose and sucrose. Total anthocyanins significantly increased during storage while ascorbic acid content remained almost unchanged in all varieties. Limonin significantly decreased in all varieties, reaching the lowest values at the end of cold storage. A consistent and significant raise of putrescine occurred during storage in the fruit of the blood varieties, while in the common orange variety no significant difference was observed. These results are in accordance to those obtained by the sensory evaluation that has shown an increase of the descriptor "off-flavour" in the last period of storage only for blood orange fruits.

S21P05

Antioxidant capacity and total phenolic contents of bergamot (*Citrus bergamia*)

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Bergamot grown in East Mediterranean Region of Turkey is cultivated mostly for its essential oil produced from its peel. Except the oil used in fragrant tea (earl grey) making and pharmaceutical industry, bergamot peel is also used in jam making. Usually only calcium citrate and citric acid are obtained from bergamot juice. With this study, some of the biochemical contents of bergamot juice were determined and its usage ways (patterns) were discussed. Two methods, namely β-carotene bleaching and DPPH (α,α-diphenyl-β-pycrylhydrazyl) assay were used to determine total antioxidant capacity, while Folin–Ciocalteu reagent was used to determine total phenols. Bergamot had high total phenolic (80.06 µg GAE/mg DW) and also high total anthocyanin content with β-carotene bleaching (60.12%) and with DPPH (180.07 mg/100ml). The study demonstrates the potential of bergamot juice for nutritional value through other fruit juices.

S21P06

Simulation of cold treatment during a cargo shipment of citrus fruits

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Commercial agreements for citrus shipment outside EU countries (Canada and USA) require both the respect of the fruit quality and the restriction of parasites spread (Medfly larvas). Both of these requirements are satisfied with the correct use of cold treatment during the shipment. This paper reports the results of a simulated refrigerated transport of citrus fruits in a 40ft container. Fruit temperature distribution in a refrigerated container (MSC 40ft) has been monitored in order to verify the temperature distribution in different positions of the load. A refrigerated container placed in the Oranfrizer s.r.l (Scordia-CT Italy) packing house, was loaded in 2 hours, starting with pallets placed in the end side of the reefer. For temperature measurements (in the air, in the

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be directly commercialized and represents a waste for producers, despite their high quality and richness in health benefits. A valid alternative is represented by the processing of outsize and defective fruits to produce juices as well as to extract high value active compounds from various parts of the fruits. In this paper the authors suggest a technological platform devoted to the recovery and valorization of PGI citrus fruits from the South of Italy in order to obtain high added value products that can be distributed and commercialized in the organic market and health industry. The technological platform could operate with the production of fresh and pasteurized citrus juice from less quality citrus fruits. The authors put their attention to the process and technology required to guarantee high quality, taste and stability of juices. They studied also the methodologies that could be used for the extraction of active compounds in mild conditions, as i.e. membrane processes or supercritical extraction, in order to recovery active substances (proteins, antioxidant, terpens) for food, pharmaceutical and chemical industries. The platform will also be used for the characterization of fruit anatomy, structure, texture and physiology features, in order to well predict the quality of fruit.

S21O06

Industrial orange juice (var. 'Salustiana') debittering: effects on sensory properties

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Debittering is an industrial process that removes bitter taste compounds in juices, which are

mainly limonoids. This process can be done by physical adsorption on a resin, which can also remove other interesting compounds related to the sensory properties. This study was aimed to analyse this effect in orange juice (OJ) of 'Salustiana' (*Citrus sinensis*). Samples (n = 9) of the industrial squeezed OJ and corresponding OJ after the debittering process (DOJ) were taken in an orange juice factory. Acidity, pH and total soluble solids were measured according to AOAC methods. No significant difference between OJs and DOJs were found. Colour was evaluated by image analysis (DigiEye System). Hue and lightness were lower (more reddish and darker) after debittering ($p<0.05$). Volatile profile (limonene, α-pinene, ethyl butanoate, octanal, linalool, citral and terpineol) were analysed by gas chromatography. All the aroma compounds decreased ($p<0.05$) (from 15.94 % to 61.16 %) after debittering as well as the total phenolic compounds measured by Folin Ciocalteu ($p<0.05$). Sensory analyses were conducted by 12 untrained panelists to determine the influence of the debittering on the perceived colour, smell and taste by paired comparison tests. The panelists did not find significant differences ($p>0.05$) in the colour, however aroma and taste were significantly ($p<0.05$) different in OJs vs DOJs.

S21P01

Anthocyanins in citrus

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Anthocyanins are water-soluble pigments belonging to the flavonoid family. Their color ranging from red to blue to purple shades according to vacuole pH, copigmentation with other polyphenols and complex with metal ions. They are synthesized by organisms of the plant kingdom and bacteria, and have been observed to occur in all tissues of higher plants, providing color in leaves, stems, roots, flowers, and fruits. Blood oranges (*Citrus sinensis*) contain anthocyanins almost exclusively in the flesh and only in some cases in the rind. As regards the distribution of anthocyanins in other citrus tissues, they are present in the young shoots and flowers of citron (*Citrus medica*) and some lemon varieties (*Citrus limon*). The same plant tissues of orange species don't accumulate anthocyanins. Therefore, the biosynthesis of anthocyanins in citrus is tissue-specific and dependent on genotype. This study aims at providing a scientific contribution to the knowledge of anthocyanins contained in fruit and other plant tissues of different citrus species. Anthocyanins of blood orange fruits ('Moro', 'Tarocco', and 'Sanguinelli') and some of their hybrids, as well as of young leaves and

flowers of different cultivars of citron and lemon were analyzed by HPLC/MS-MS. The dominant pigments in fruits were cyanidin-3-glucoside and cyanidin-3-(6" malonil-glucoside) while in young leaves and flowers a different pattern was observed. This is the first report of patterns of anthocyanins in the different organs of a large number of genotypes of citrus.

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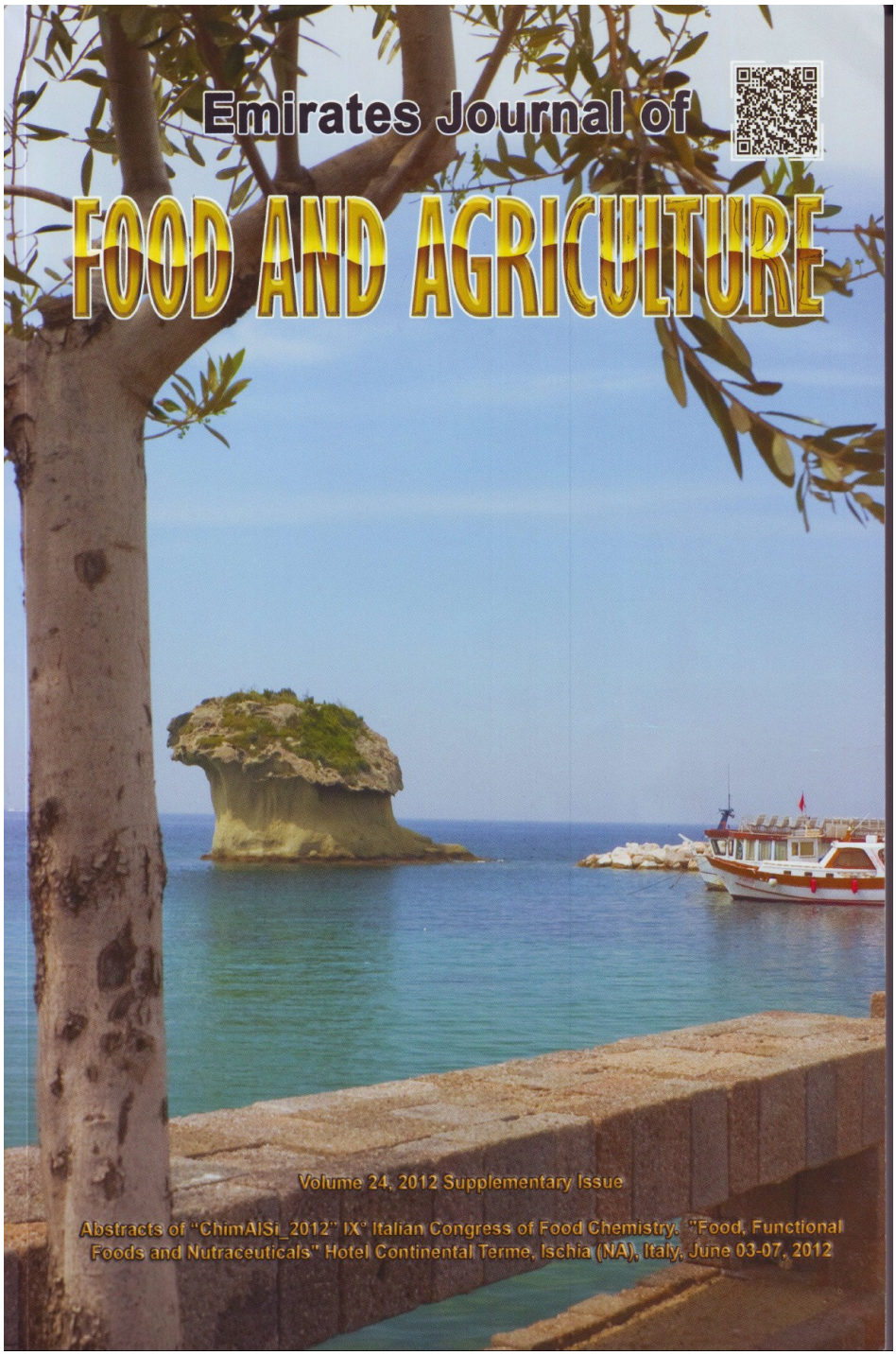
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P-178

New triploid citrus hybrids: quality and functional properties of fruits

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Abstract
Nowadays the crossbreeding of the best existing *Citrus* species is an important strategy for producing new valuable hybrids. Since 1978, the CRA-Centro di Ricerche per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM) has been working on a genetic improvement program to develop new seedless *Citrus* hybrids. The development of triploid hybrids by crossing a monoembryonic *2x* female parent with a *4x* male parent has been the successful breeding strategy carried out by the CRA-ACM. Within this program three new triploid hybrids have been recently obtained, namely 'D2238', diploid 'Monreal' clementine (*Citrus clementina* Hort. ex Tan.) x tetraploid 'Duncan' grapefruit (*Citrus paradisi* Macf.), 'C2710', diploid 'Oroval' clementine (*C. clementina* Hort. ex Tan.) x tetraploid 'Tarocco' orange (*C. sinensis* L. Osbeck) and 'RC1', diploid 'Fantastico' bergamot (*C. bergamia* Risso) x tetraploid 'Tarocco' orange (*C. sinensis* L. Osbeck). In order to investigate the heritability of traits from their parents, the fruit juices of these hybrids were analyzed to evaluate parameters related to fruit quality, as well as the content of health-promoting components such as ascorbic acid, flavanones, anthocyanins (in 'C2710') and hydroxycinnamic acids. In addition, the total antioxidant capacity of the juices was measured *in vitro* by the DPPH scavenging activity assay. Results showed that 'D2238' hybrid presented fruit quality characteristics intermediate to those from both parents, being morphologically similar to grapefruit, with some of the valuable characteristics of clementine (low acidity, ease of peeling, excellent juice yield) and a moderate content of naringin (~ 250 ppm), a trait inherited from the male parent. The 'C2710' hybrid was similar in shape and size to a large clementine with some excellent traits inherited from the 'Tarocco' orange, such as organoleptic properties (juiciness, sugar/acid ratio), ascorbic acid content, and a red flesh pigmentation due to the presence of anthocyanins. The 'RC1' fruits resembled 'Fantastico' bergamot respect to physico-chemical parameters (relevant peel thickness, low juice yield, high acidity) and presented a original flavanone profile with the presence of neoeriocitrin, typically present in bergamot juice but not in sweet oranges, as well as narirutin and esperidin. IC_{50} values (µl of juice yielding 50% inhibition of DPPH) for the three hybrids ('RC1'>'D2238'>'C2710') reflected the concentration of bioactive compounds in their juice. Although these three new hybrids don't show great potentiality to be consumed as fresh fruit, they can be considered as a new valuable and original source of natural antioxidants to be exploited for processing.

P-179

Tracking wine adulteration: Identification of barbera grape in Nebbiolo wines using microsatellite DNA analysis (SSR)

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Abstract
Wine traceability/authentication is a key target for Italy, the world's second largest wine producer behind France. Several European Countries developed appellation systems, with their own unique labels and seals, to try to combat label fraud that misrepresented a wine's true origins. The fraudulent use of low-price grapes/musts/wines to adulterate high-value wines (particularly when wine is produced in purity, using a unique grape variety, like Barolo and Barbaresco DOCG wines from Piedmont, from Nebbiolo grape) has been highlighted in Italy. The fraudulent substitution of Brunello di Montalcino wine is a key example. Wine experts suspect that, as much as 5% of the wine sold in secondary markets worldwide, could be counterfeit. Despite the high number of methods for the traceability of wine using complex, time consuming and expensive techniques (e.g. SNIF-NMR, stable isotope ratio mass spectrometry, trace elements), few applications reported the use of DNA analysis in bottled wine (grape and must are largely studied). The polymorphism of SSR (Single Sequence Repeats or microsatellites, regions of tandem repeats of two to five nucleotides that are ubiquitous in eukaryotic genomes) is sufficiently stable to be used in genetic analyses. Considering the low quantity of DNA in wine (depeding on the impact of processing, particularly filtration and refining steps) as well as the interference of polyphenols both during DNA extraction and DNA amplification steps, a key strategic problem is correlated with the extraction

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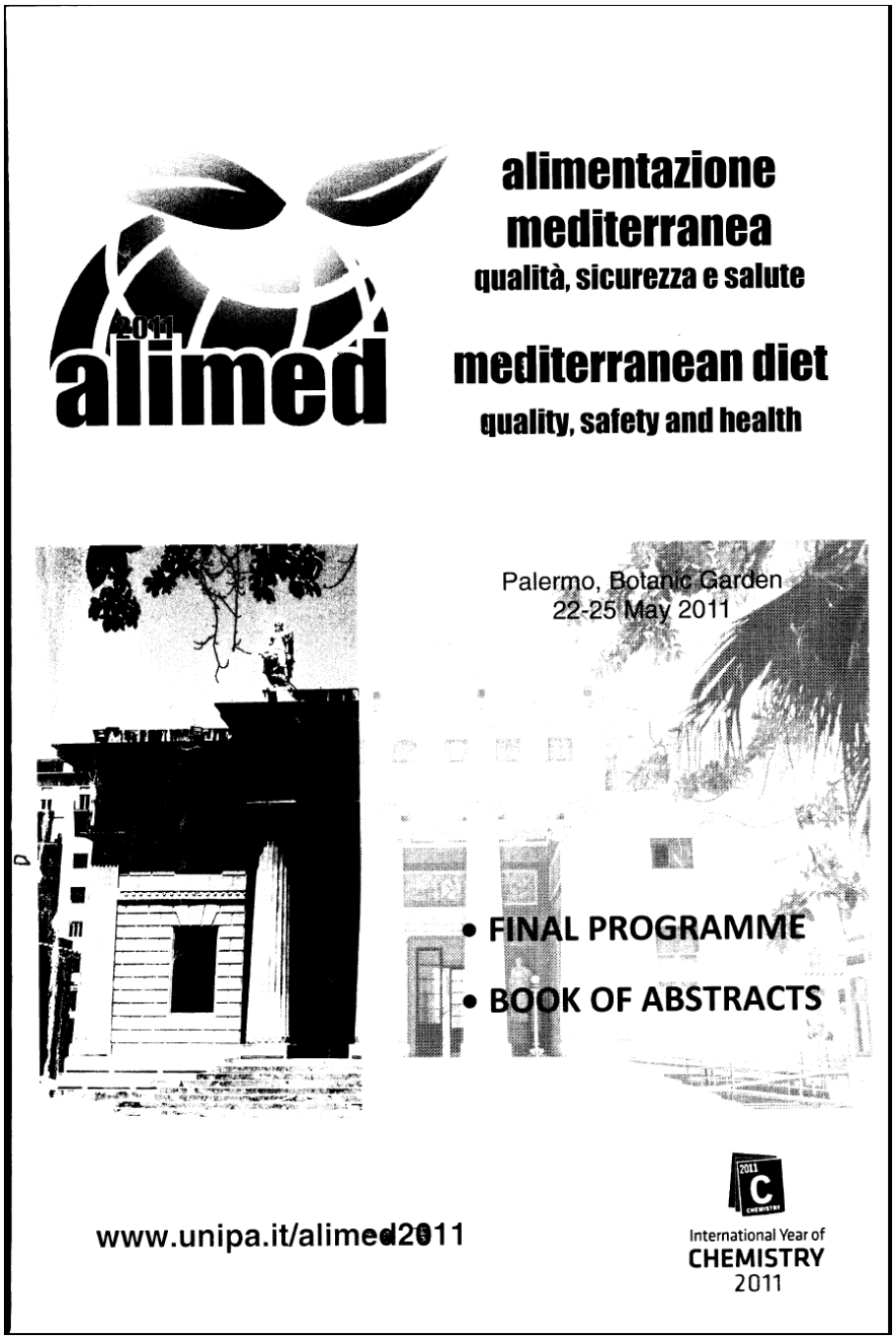
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CHANGES IN SENSORY QUALITY OF BLOOD AND COMMON ORANGE FRUITS DURING COLD STORAGE

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Orange fruits may undergo changes in sensory quality during prolonged cold storage, probably associated with decreased levels of characteristic aroma compounds present in fresh fruits, as well as with development of off-flavours. The purpose of this work was to evaluate changes of components related to aroma of two blood varieties (cv. Tarocco and Moro) and a common variety (cv. Washington navel) of orange fruits [*Citrus sinensis* (L.) Osbeck] stored at 6 ± 1 °C and 90-95 % RH for 60 days. Juice quality parameters (TSS total soluble solid, TA titratable acidity, pH) in addition to individual sugars (glucose, fructose and sucrose), ascorbic acid, total anthocyanins, putrescine and limonin were determined during fruit storage. Moreover, sensory assessments were carried out by a trained sensory panel at 15 days intervals. TSS, TA and pH did not show significant variations during storage in all varieties. Such a trend was confirmed by evaluating glucose, fructose and sucrose. Total anthocyanins significantly increased during storage while vitamin C content remained almost unchanged in all varieties. Limonin significantly decreased in all varieties, reaching the lowest values at the end of cold storage. In fruit of the blood varieties a consistent and significant increase of putrescine occurred during storage, while in the common orange variety no significant difference was observed. These results are in accordance to those obtained by the sensory evaluation carried out by a trained panel that has shown an increase of the descriptor "off-flavour" in the last period of storage only for blood orange fruits.

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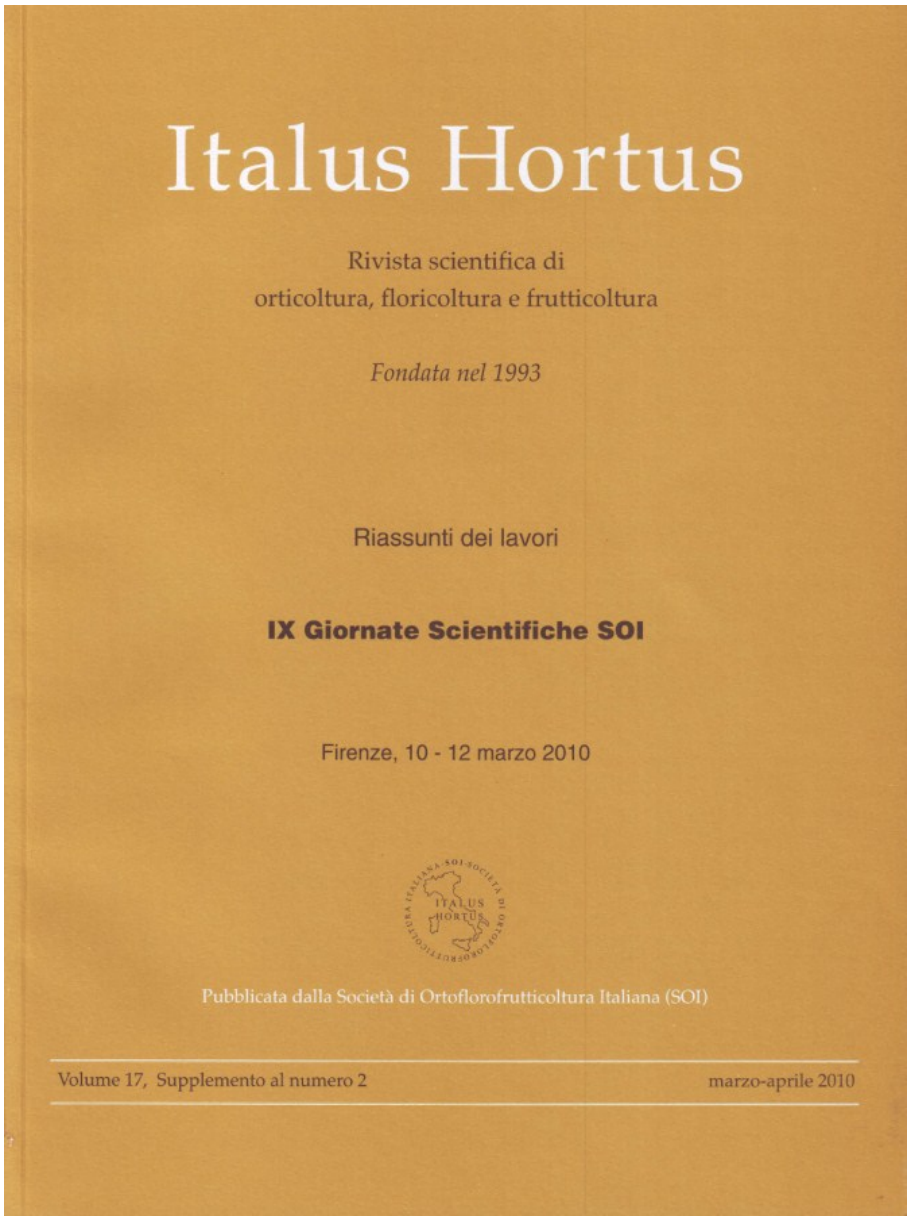

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IX Giornate Scientifiche SOI

Firenze, 10 - 12 marzo 2010

Pubblicata dalla Società di Ortoflorofrutticoltura Italiana (SOI)

Volume 17, Supplemento al numero 2 marzo-aprile 2010

Valutazione di otto portinesti innestati con 'Nagami' kumquat infetto da *Citrus leaf blotch virus* (CLBV)

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Citrus leaf blotch virus, virus appartenente alla famiglia dei flexiviridae, è stato trovato associato alla disaffinità d'innesto del "Nagami" Kumquat [*Fortunella margarita* (Lour.) Swingle] con i portinnesti trifogliati.

Scopo del lavoro è stato valutare il comportamento di questa specie in diverse combinazioni d'innesto.

Sono state innestate marze di "Nagami" Kumquat infette dall'isolato CLBV ISA 9-ME-I su 8 portinnesti diversi: poncirus [*Poncirus trifoliata* (L.) Raf.)], poncirus "Flying Dragon" [*P. trifoliata* var. monstrosa (T. Ito) Swingle], citrumelo "Swingle" (*P. trifoliata* x *Citrus paradisi)*, limone "Volkameriano" (*C. volkameriana* Tan. e Pasq.), *C. macrophylla* Wester, arancio amaro (*C. aurantium* L.), citrange "Troyer" e "Carrizo" (*P. trifoliata* x *C. sinensis*).

La prova è stata condotta in un vivaio nella provincia di Messina. Le marze sono state innestate nel mese di maggio 2007. Per ogni combinazione d'innesto sono state effettuate 16 repliche e le piante sono state allevate secondo le pratiche colturali vivaistiche.

Dopo circa 40 giorni è stato verificato l'attecchimento delle marze e periodicamente sono stati effettuati i controlli sulla crescita e sui sintomi indotti dall'infezione. Dopo 12 mesi tutte le piante sono state saggiate per verificare la presenza del CLBV. Inoltre sono state condotte osservazioni finali a 2 anni dall'innesto.

Le osservazioni effettuate sull'attecchimento dell'innesto ha evidenziato che è stato necessario reinnestare un numero di marze maggiore sui portinnesti trifogliati. In particolare il citrange "Troyer" è stato il portinnesto su cui è stato effettuato il numero più alto di reinnesti (10/16) seguito dal citrumelo "Swingle" (7/16) e dal citrange "Carrizo" (7/16); invece, su *C. macrophylla* e su arancio amaro è stato effettuato soltanto un reinnesto.

Dopo 12 mesi circa, le piante sono state saggiate tramite RT-PCR usando primers specifici per la proteina capsidica e per le RNA polimerasi. I saggi hanno confermato l'infezione di CLBV su tutte le piante saggiate tranne su sei, due innestate su a. amaro e una rispettivamente su poncirus, citrange "Carrizo", citrange "Troyer" e poncirus "Flying Dragon".

Il numero maggiore di perdite è stato osservato sui portinnesti trifogliati con una percentuale massima del 75% su Poncirus "Flying Dragon" e più bassa sugli altri portinnesti: citrumelo (56,25%), citrange "Carrizo" (50%), citrange "Troyer" e poncirus (43.75%). Le piante rimaste hanno presentato uno sviluppo ridotto, fogliame molto clorotico e lieve disaffinità d'innesto.

Sul limone "Volkameriano" è stata osservata una perdita del 18.7% mentre su *C. macrophylla* e arancio amaro una perdita del 6.25%. Inoltre, su questi ultimi portinnesti non è stato osservato alcun sintomo della malattia e lo sviluppo della chioma è stato normale.

Dopo due anni dall'innesto i risultati hanno confermato la suscettibilità dei portinnesti trifogliati al CLBV, avendo avuto la maggior percentuale di perdita delle piante. Le combinazioni con arancio amaro, *C. macrophylla* e il limone "Volkameriano" si sono dimostrati tolleranti non avendo mai mostrato alcun sintomo della malattia. Pertanto, in attesa della disponibilità di materiale di propagazione risanato di "Nagami" Kumquat è opportuno l'utilizzo di questi ultimi portinnesti per la produzione commerciale di questa specie.

Qualità e aspetti salutistici di frutti di clementine biologici e convenzionali

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Negli anni recenti si è registrata una domanda crescente di prodotti provenienti da agricoltura biologica perché considerati dai consumatori più sicuri e salutistici di quelli ottenuti con metodi convenzionali. I prodotti biologici, a differenza di quelli ottenuti in regime convenzionale, sono solitamente esposti a stress biotici che inducono l'accumulo di composti fenolici e quindi differenze nel contenuto di componenti dotati di attività antiossidante. La presente ricerca ha avuto come obiettivo la caratterizzazione qualitativa di frutti di clementine (*Citrus clementina* Hort. Ex Tan.) biologici e convenzionali volta ad evidenziare le proprietà nutrizionali e salutistiche delle produzioni biologiche, utili per la valorizzazione e promozione del prodotto. In particolare, lo studio è stato condotto in un periodo di tre anni (2006-2008) su frutti di clementine cv. "Comune" provenienti da 9 aziende agrumicole gestite da almeno tre anni, in regime biologico e altrettante condotte con metodi convenzionali. Le suddette aziende, localizzate in aree vocate della Calabria, sono state selezionate in modo da presentare omogeneità rispetto all'età e ai portinnesti impiegati. I frutti biologici e convenzionali sono stati caratterizzati valutando i parametri classici della qualità (peso medio frutti, resa in succo, solidi solubili totali, acidità totale, pH) ed altri indici, importanti per la loro attività biologica, quali il contenuto di acido ascorbico, di polifenoli totali e l'attività antiossidante totale determinata *in vitro* mediante saggio ORAC. Tra i parametri fisico-chimici determinati il peso frutti, i solidi solubili totali, l'acidità totale ed il rapporto di maturazione hanno mostrato differenze statisticamente significative, con valori più elevati nei campioni biologici rispetto ai convenzionali. Il contenuto di acido ascorbico e polifenoli totali e l'attività antiossidante, espressa in unità ORAC, sono risultati significativamente più elevati ($p \leq 0{,}001$) nei frutti di clementine biologici rispetto ai convenzionali. Questi risultati hanno permesso di evidenziare le spiccate proprietà antiossidanti e salutistiche dei prodotti coltivati in regime biologico. Tale aspetto assume un'importanza fondamentale perché fornisce ai produttori, che si trovano oggi a fronteggiare il declino dei prezzi dei prodotti convenzionali, la possibilità di dare valore aggiunto alle proprie produzioni biologiche.

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Authentication of the Geographical Origin of Sicilian Typical Citrus Fruits through a Chemical and Chemometric Approach

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Abstract

Sicilian citrus industry is currently involved in a marked crisis mainly related to the lack of a commercial strategy along the whole chain of production. This crisis can be overcome focusing on typical citrus productions, which have been awarded PGI (Protected Geographical Indication) recognition by the European Commission. Thus it comes the need of a system of traceability to verify the authenticity of these productions. The aim of this research was to classify, by a physico-chemical and chemometric approach, two Sicilian typical citrus productions ('Arancia Rossa di Sicilia' and 'Limone di Siracusa') whose geographical origin was authenticated by samplings made in PGI or not-PGI areas. The combined use of physico-chemical (quality parameters), spectral (NIR spectral patterns), multielemental (Fe, Zn, Mn, Cu, Li, Sr) and isotopic ($^{13}C/^{12}C$, $^{18}O/^{16}O$) markers joint with

multivariate statistical analysis such as PCA (Principal Component Analysis) and LDA (Linear Discriminant Analysis) was used as a tool to discriminate between PGI and not-PGI Sicilian typical citrus fruits. The results have revealed that a representative database of each geographical area can be used for the development of a traceability system of such typical productions.
Keywords: *Citrus sinensis* L. Osbeck, *Citrus limon* (L.) Burm.F., orange fruit, lemon fruit, NIR, isotope ratio, multielemental composition, traceability, multivariate analysis

Introduction

Sicily is characterized by a natural vocation toward the cultivation of citrus, as evidenced by the wide number of species and varieties cultivated in the region.

As concerns sweet oranges, Tarocco, Moro and Sanguinello blood (pigmented) oranges are the most cultivated varieties in Sicily, although in the last two decades the Tarocco cultivar is the most widespread variety in Sicily. Blood oranges are mainly grown in the eastern part of Sicily on the southern mountainsides of the Etna volcano, an area bounded among Catania, Enna and Siracusa districts. The fruits of these varieties are well appreciated on national and foreign markets for their organoleptic, nutritional and healthy properties, distinctive qualities that led the European Union to grant the PGI (Protected Geographical Indication) recognition to the 'Arancia Rossa di Sicilia' in 1996 (EC Reg. 1107/1996). Also the lemon grown in Sicily presents some varietal excellences whose cultivation is primarily located in areas where the pedo-climatic conditions allow the fruit to reach high quality standards. In particular, the 'Femminello siracusano' lemon fruit, appreciated for the high acidity and the juice yield, has recently been recognized as PGI "Limone di Siracusa" (EC Reg. 96/2011).

Despite the high quality of the citrus productions, Sicilian citrus industry is currently involved in a marked crisis mainly related to the lack of a commercial strategy along the whole chain of production. A way to overcome this crisis can be represented by the valorisation of Sicilian typical citrus productions which have been awarded PGI recognition by the European Commission. It is possible, through the implementation of a reliable system of traceability to verify the authenticity of these productions and the effective compliance to production regulations, thus avoiding frauds and ensuring consumer protection. The increased demand for typical products has raised the question of the authenticity of foods which carry one of the European certified labels (PDO-Protected Designation of Origin, PGI-Protected Geographical Indication, TSG-Traditional Specialty Guaranteed), thus driving the scientific research towards the identification of new analytical techniques to be employed to classify and verify the origin and the authenticity of these typical productions.

In food provenance studies, it is important to consider all the factors which may have an influence on the composition and the overall characteristics of the product. Thus it is possible to use several investigation tools, such as chemical and spectroscopic variables, in order to exploit the information related to different aspects of the intrinsic nature of a food product. Chemical methods have been applied mainly for the evaluation of classical, nutritional and health quality parameters and, in recent years, they have been standardized in officially accepted procedures. NIR (Near InfraRed) spectroscopy is considered as a useful method for the evaluation of the authenticity of the geographical origin of a food (Manley et al. 2008). This technique is based on the absorption of electromagnetic radiations in the near-infrared region of the electromagnetic spectrum (from 780 to 2.500 nm or from 12.820 to 4.000 cm^{-1}). The NIR absorption bands which constitute the characteristic spectrum of a food sample (fingerprint)

are attributable to the combination of fundamental vibrations relative to specific functional groups of the biomolecules. NIR spectra present extremely complex variations with overlaying and wide bands, therefore the information provided by the spectra acquisition must be processed through multivariate chemometric tools. Multielement profiling is based on several environmental and geologic factors such as soil type, rainfall, and temperature of a growing region and provides a scientific underpinning to determine the geographic origin of a commodity (Perez et al., 2006). Early investigations used atomic absorption to determine elemental concentrations. The introduction of inductively-coupled plasma-optical emission spectrometry (ICP-OES) allowed a wider range of elements to be analysed. These large number of trace elements together with chemometric software packages have been successfully used to monitor quality, authenticity and country of origin of orange juices (Simpkins et al., 2000). Isotope ratios have been used as another chemical profiling method to determine the geographical origin of different foodstuffs of vegetable or animal origin. Many natural phenomena, classed as physicochemical effects, can lead to isotope fractionation, producing changes in the ratio of the 'heavy' to 'light' isotope of a stable element. These differences are, at natural abundance levels, relatively small, thus isotope ratio analysis through Isotope Ratio Mass Spectrometry (IRMS) is achieved comparing the ratio of stable isotopes in the sample to a reference compound of nominal isotope ratio, both expressed in delta units (‰, per mil). The relative proportions of the natural abundance of isotope ratios have been usefully employed for food provenance determinations. The measurement of the stable isotope ratios of hydrogen and oxygen are applicable to the characterization of geographical origin because they are strongly latitude dependent. Moreover, there is a gradient of decreasing $^{13}C/^{12}C$ in plant material

from the equator to the poles which can also be used as a proxy for geographical origin determination (Kelly et al., 2005).

The aim of this research was to develop a system for the certification of the geographical origin of two Sicilian typical citrus products such as PGI 'Arancia Rossa di Sicilia' and PGI 'Limone di Siracusa', through the joint use of physicochemical parameters, spectral (NIR spectral patterns), multielemental (Fe, Zn, Mn, Cu, Li, Sr) and isotopic ($^{13}C/^{12}C$, $^{18}O/^{16}O$) variables. In addition, multivariate statistical techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA) were used in order differentiate between PGI and non-PGI citrus fruits.

Materials and methods

Plant material

The study was conducted over a two year period (2011-2012) on orange fruits (cv. Tarocco, Moro and Sanguinello) collected at maturity from several farms located either inside the boundaries of the production zone of the PGI 'Arancia Rossa di Sicilia' in the citrus area of eastern Sicily (n=105), and outside the PGI area (non-PGI) (n=17) in Sicilia, Calabria, Basilicata, and Sardegna regions. In each farm samples of 50-60 fruits were harvested, at commercial maturity (TSS/AT > 8); from 5-10 trees from the outer part of the canopy and from the four cardinal points. Each sample was divided into three subsamples and on each of these chemical (quality parameters), spectral (NIR spectral patterns), multielemental (Fe, Zn, Mn, Cu, Li, Sr) and isotopic ($^{13}C/^{12}C$, $^{18}O/^{16}O$) variables were determined.

Within the same period, lemon fruits cv. "Femminello siracusano" were collected at maturity (the winter crop 'Primofiore') from several farms located either inside the boundaries of the production zone of the PGI 'Limone di Siracusa' (n=21) and outside the PGI area (non-PGI) (n=44) in Sicilia and Campania regions. Two samples of lemon

fruits coming from Spain and Argentina were even purchased in a local market. In each farm samples of 50-60 fruits were harvested, at commercial maturity from 5-10 trees from the outer part of the canopy and from the four cardinal points. Each sample was divided into three subsamples and on each of these physicochemical (quality parameters), spectral (NIR spectral patterns), multielemental (Fe, Zn, Mn, Cu, Li, Sr) and isotopic ($^{13}C/^{12}C$, $^{18}O/^{16}O$) parameters were determined.

Fruit quality

Titratable acidity (TA), pH, total soluble solids (TSS), fruit weight and juice yield were determined according to conventional methods (Kimbal, 1991). The ascorbic acid concentration was evaluated by liquid chromatography using a Waters Alliance 2695 HPLC (Waters Corporation, Milford, MA) equipped with a Waters 996 photodiode array detector and Empower software (Rapisarda et al., 1996). Briefly, 5 mL of centrifuged juice was poured into a flask and made up to 50 mL with 3% metaphosphoric acid solution. An aliquot of the solution was 0.45 μm filtered, and 20 μL injected into the HPLC. The mobile phase was 0.02 M H_3PO_4 and the detector was set at 260 nm. Total anthocyanin content was determined spectrophotometrically (Varian UV–vis spectrophotometer, model Cary 100 Scan; Varian Inc., Palo Alto, CA) by the pH differential method (Rapisarda et al., 2000) and expressed as cyanidin 3-glucoside equivalents (mg/L).

FT-NIR measurements

FT-NIR spectra were obtained according to Rodriguez-Saona et al.(2001), with some modifications. In brief, 600 μL of juice were poured on a glass microfibre filter disk (Whatmanglass microfibre filter Ø 25 mm) placed inside a Petri dish base (Ø 25 mm). The samples were dried in a ventilated oven for 24 hs at 35 ± 1°C. Then FT-NIR spectra were recorded using a Spectrum 100 N (Perkin Elmer, Waltham, Massachussets) spectrometer from 10.000 to 4.000 cm^{-1} ,

operating at intervals of 2 cm^{-1}. Measurements were acquired operating in reflectance mode by using the reflectance accessory. The total number of data points was 3001 for each spectrum.

Multielement analysis

Juice samples were analyzed for Fe, Zn, Mn, Cu, Li, Sr content (mg/ L) by Inductively Coupled Plasma Spectrometry (ICP-OES Optima 2000DV, Perkin Elmer, Italy). Centrifuged juice samples were subjected to dry digestion in muffle furnace at 550 °C for 20 hs. Then samples were solubilised with a solution containing 4 mL of distilled water and 0.5 mL of concentrated nitric acid. The solutions were poured into a flask and made up to 50 mL with distilled water before measurements.

Stable isotope ratio analysis

All of the samples were subjected to the analysis of the $^{13}C/^{12}C$ in the juice using an isotope ratio mass spectrometer (Delta plus XP ThermoFinnigan, Bremen, Germany) equipped with an elemental analyzer (EA Flash 1112 ThermoFinnigan). The $^{18}O/^{16}O$ ratio of juice water was analyzed in CO_2 according to the water equilibration method described in the ENV 12141 method. The values were expressed in δ‰ against international standards (Vienna-Pee Dee Belemnite for $\delta^{13}C$, Vienna-Standard Mean Ocean Water for $\delta^{18}O$). The isotopic values were calculated against working in-house standards and calibrated against international reference materials including sugar IAEA-CH-6 (IAEA) for $^{13}C/^{12}C$.

Statistical analysis

Statistical analysis of the results was carried out using the STATSOFT 6.0 program (Vigonza, Padova, Italy). The statistical differences were evaluated by variance analysis (ANOVA), and means separation was conducted using the Tukey test. A multivariate statistical approach have been carried out through Principal Component Analysis (PCA)

and Linear Discriminant Analysis (LDA) by using the SPSS 18.0 software (IBM, 2009).

Results and discussion

Table 1 shows results of physicochemical parameters, including ascorbic acid and total anthocyanins content, in blood orange fruits sampled in PGI and in non-PGI areas. Fruit weight, juice yield and TSS showed significantly higher values ($p \leq 0.01$) in PGI samples respect to those sampled outside the PGI production area. Even the total anthocyanin content was significantly greater ($p \leq 0.001$) in the fruits sampled inside the boundaries of the production zone of the PGI 'Arancia Rossa di Sicilia'. However, the production area showed no influence on the values of pH and TA of the fruits and on the ascorbic acid content. Stable isotope ratios ($\delta^{13}C$, $\delta^{18}O$) and multielemental composition (Fe, Zn, Mn, Cu, Li, Sr) showed no statistically significant difference. (Table 2). The NIR spectra of the PGI and non-PGI blood orange juices (Figure 1), acquired in the spectral range between 10.000 and 4.000 cm-1, have allowed to collect, for each sample, 3001 absorbance values recorded at intervals of 2 cm^{-1}. The application of this technique produces complex spectra, which are very difficult to analyze because of the large number of data points (absorbance values at intervals of 2 cm^{-1}). The Principal Component Analysis (PCA) is a statistical method of multivariate analysis that reduces the dimension of a complex dataset composed by a large number of highly correlated variables, and replace them with a fewer number of new variables (principal components) not correlated one another while linearly correlated to the original variables. The application of PCA has allowed to reduce the dimension of the spectral data in a small number of components (5 principal components) that explained 99.98% of the variance of the original data. The factor scores of the 5 principal components were then used, together with the physicochemical, multielemental and isotopic

parameters, as variables in a Linear Discriminant Analysis (LDA) model. The LDA is a classification technique which assumes that p variables (quantitative variables) were measured on observations belonging to two or more groups with the aim of finding one or more linear combinations which allow a good discrimination between the various groups. The LDA standard principle for the selection of the latent variables consist in maximising differences between groups and lowering the variance within groups. The method produces (n-1) orthogonal linear discriminant functions, with n = number of the groups, that allow the samples to be classified into one or another group. In our research the linear combination of the considered variables allowed us to evaluate the possibility of differentiating the PGI blood orange fruits respect to the non-PGI ones (groups). The LDA generated one discriminant function, highly significant at Wilks' Lambda test ($p \leq 0.0001$), which provided a good discrimination between the two groups. In figure 2 a graphical representation of the discriminant scores assigned by the LDA model to the observed cases respect to their frequencies is reported. The standardized canonical discriminant function coefficients showed that the parameters which mostly contributed to the differentiation between the two groups were juice yield (0.605), TSS (0.840) and total anthocyanin content (0.389). With regard to classification results 75.9% of cases were correctly classified as PGI and 88,2% as non-PGI.

The production area of the PGI ‘Arancia Rossa di Sicilia’ includes four districts (Catania, CT; Siracusa, SR; Ragusa, RG; Enna, EN) located in the eastern part of Sicily on the southern mountainsides of the Etna volcano. In order to assess whether a discrimination between PGI blood orange fruit collected in the different districts within the PGI area could be achieved, the same statistical methodology have been applied with the aim of evaluating the existence of homogeneous groups within the aforesaid area. In particular, the collected data

(physicochemical, NIR, multielemental and isotopic variables) have been processed through a LDA model. The linear combination of the considered variables produced three canonical discriminant functions which explained the 88,9% of the variance of the original data. The score plot of the first two discriminant functions (figure 3) showed that function 1, which explained 63.6% of the variance, allow a quite clear discrimination between the samples collected in RG from those collected in EN and a slight differentiation from the other two grouped districts (SR and CT), while respect to function 2 (25.3% of variance), all the cases are overlapping. Thus it can be concluded that a discrimination between blood orange fruits sampled in the four different districts within the same PGI area cannot be achieved.

The results of the physicochemical determinations and the ascorbic acid content in lemon fruits cv. 'Femminello siracusano' sampled in PGI and in non-PGI areas are shown in Table 2. Among the determined parameters, juice yield, TA and TSS showed statistically significant differences ($p \leq 0.01$), with higher values in the samples collected in the non-PGI area. However the production area showed no influence on the values of fruit weight and ascorbic acid content. Multielemental composition and stable isotope ratios in lemon fruits cv. 'Femminello siracusano' sampled in PGI and in non-PGI areas are reported in table 4. The oxygen isotope ratio $\delta^{18}O$ ($p \leq 0.01$), Mn ($p \leq 0.01$) and Sr contents ($p \leq 0.05$) showed statistically significant differences, with higher values found in samples from the non-PGI production area, while $\delta^{13}C$ and Fe, Zn, Mn, Cu, Li contents showed no statistically significant difference. As reported for blood orange fruits, NIR spectra of the PGI and non-PGI lemon juices (Figure 1), acquired in the spectral range between 10.000 and 4.000 cm-1, have allowed to collect, for each sample, 3001 absorbance values which were used as variables in a preliminary PCA. The factor scores of the 5 principal components derived from the PCA, which explained the

99.98% of the variance of the original data, were then used, together with the physico-chemical, multielemental and isotopic parameters, as new variables in a LDA model in order to evaluate the possibility to differentiate the PGI productions from the non-PGI ones. The LDA generated one discriminant function highly significant at Wilks' Lambda test ($p \leq 0.0001$), which provided a good discrimination between the two groups. Figure 4 shows a graphical representation of the discriminant scores assigned by the LDA model to the observed cases respect to their frequencies. The classification results showed that 85% of cases were correctly classified as PGI and 80,2% as non-PGI. The analysis of the standardized canonical discriminant function coefficients showed that the parameters that mostly influenced the discrimination between the two groups were $\delta^{18}O$ (0.750), Mn content (0.526) and pH (-0.515).

Conclusions

The results of our study demonstrated that the joint use of physicochemical (quality parameters), spectral (NIR spectral patterns), multielemental (Fe, Zn, Mn, Cu, Li, Sr) and isotopic ($^{13}C/^{12}C$, $^{18}O/^{16}O$) parameters is functional for the development of a system for the certification of the geographical origin of citrus fruit produced in PGI areas. Moreover the combined chemometrical approach is a valid tool for traceability studies in order to authenticate the geographical origin of the two Sicilian PGI productions ‘Arancia Rossa di Sicilia’ and ‘Limone di Siracusa’. In particular, it has been demonstrated that a high percentage of the grouped cases has been correctly classified, (82.0% for blood orange fruits; 82.6% for lemon fruits). Thus in both cases, the method applied in this study explained a clear discrimination between fruits collected in farms located within the PGI area from those collected in non-PGI zones.

References

KELLY S., HEATON K., HOOGEWERFF J. 2005. *Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis*. Trends in Food Science & Technology, 16: 555-567.

KIMBAL D.,1991. *Citrus Processing. Quality Control and Technology*. AVI Books (New York).

MANLEY M., DOWNEY G., BAETEN V. 2008. *Spectroscopic Technique: Near-infrared (NIR) Spectroscopy*. In Modern Techniques for Food Authentication. Da-Wen Sun Ed. Academic Press, Park Avenue South, New York, USA: 65-115.

PEREZ A. L., SMITH B. W., ANDERSON K. A. 2006. *Stable Isotope and Trace Element Profiling Combined with Classification Models To Differentiate Geographic Growing Origin for Three Fruits: Effects of Subregion and Variety.* J. Agric. Food Chem., 54: 4506-4516.

RAPISARDA P., INTELISANO S., 1996. *Sample preparation for vitamin C analysis of pigmented orange juices*. Ital. J. Food Sci.,3: 251-256.

RAPISARDA P., FANELLA F., MACCARONE E., 2000. *Reliability of analytical method for determining anthocyanins in blood orange juice.* J. Agric. Food Chem., 48: 2249-2252.

RODRIGUEZ-SAONA L.E., FRY F.S., MCLAUGHLIN M.A., CALVEY E.M., 2001. *Rapid analysis of sugars in fruit juices by FT-NIR spectroscopy.* Carbohydrate Research, 336: 63-74.

SIMPKINS A. W., LOUIE H., WU M., HARRISON M., GOLDBERG D., 2000. *Trace elements in Australian orange juice and other products*. Food Chemistry, 71, 423-433.

Table 1 Physico-chemical parameters, ascorbic acid and total anthocyanins content in blood orange fruits sampled in PGI and in non-PGI areas.

Area of production	Fruit weight (g)	Juice yield (%)	pH	TA[a] (%)	TSS[a] (%)	AA[a] (mg/100 mL)	Total anthocyanins (mg/L)
PGI	192,19 A	48,77 A	3,64	1,23	11,71 A	60,76	48,78 A
NOT-PGI	223,50 B	42,95 B	3,65	1,17	10,82 B	60,10	18,84B

$p \leq 0{,}01$

[(a)] TA: Total Acidity; TSS: Total Soluble Solids; AA: Ascorbic Acid

Table 2 Multielemental composition and stable isotope ratios in blood orange fruits sampled in PGI and in non-PGI areas.

Area of production	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	Li (mg/L)	Sr (mg/L)
PGI	0,83	-25,25	1,07	0,77	0,11	0,45	2,57	0,38
NOT-PGI	0,68	-26,07	1,00	0,62	0,11	0,44	3,08	0,34

Table 3 Physico-chemical parameters and ascorbic acid content in lemon fruits cv. 'Femminello siracusano' sampled in PGI and in non-PGI areas.

Area of production	Fruit weight (g)	Juice yield (%)	pH	TA[a] (%)	TSS[a] (%)	AA[a] (mg/100 mL)
PGI	143.86	33.83 B	3.07 A	5.93 B	7.67 B	42.34
NOT-PGI	143.72	36.67 A	3.01 B	6.40 A	8.10 A	42.39

$p \leq 0{,}01$

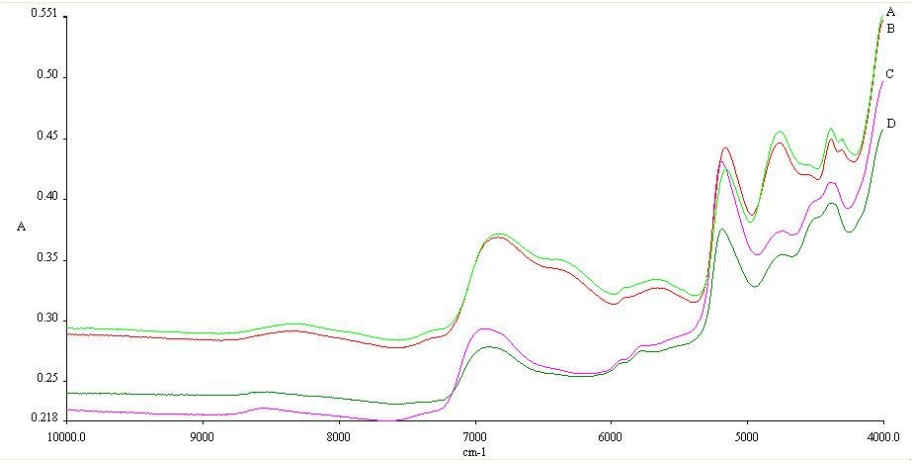
[(a)] TA: Total Acidity; TSS: Total Soluble Solids; AA: Ascorbic Acid

Table 4 Multielemental composition and stable isotope ratios in lemon fruits cv. 'Femminello siracusano' sampled in PGI and in non-PGI areas.

Area of production	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	Li (mg/L)	Sr (mg/L)
PGI	-0.74 B	-25.72	0.75	0.72	0.11 B	0.49	2.16	0.25 b
NOT-PGI	0.04 A	-25.81	1.26	0.79	0.13 A	0.54	2.60	0.32 a

Capital letters: $p \leq 0{,}01$; small letters: $p \leq 0{,}05$

Figure 1 NIR spectra of randomly-selected 'Tarocco' orange and 'Femminello siracusano' lemon fruits sampled in PGI and non-PGI areas



(A) 'Tarocco' orange juice, non-PGI; (B) 'Tarocco' orange juice, PGI; (C) 'Femminello siracusano' lemon juice, PGI; (D) 'Femminello siracusano' lemon juice, non-PGI

Figure 2 Differentiation of the production area in blood orange fruits as represented by the scores of the discriminating function generated by the LDA.

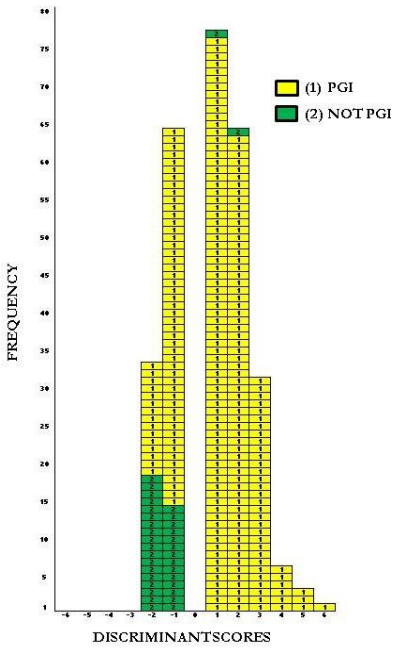


Figure 3 Differentiation between the districts inside the PGI area in blood orange fruits as represented by the scores of the discriminating functions generated by the LDA.

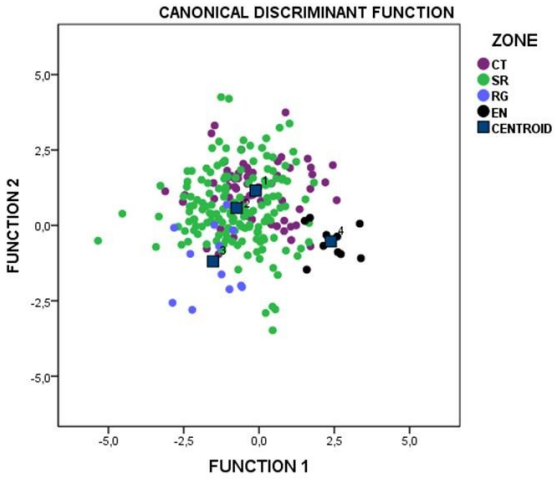
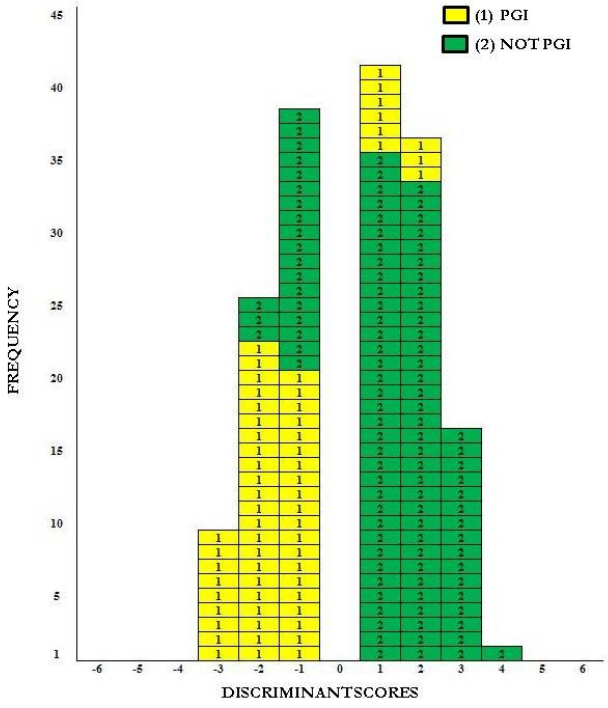


Figure 4 Differentiation of the production area in lemon fruits cv. 'Femminello siracusano' as represented by the scores of the discriminating function generated by the LDA



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XII INTERNATIONAL CITRUS CONGRESS
VALENCIA / SPAIN
BOOK OF ABSTRACTS
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S21O01

Biochemical and molecular mechanisms involved in mandarin flavor deterioration after harvest

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The sensory preference of mandarins declines during storage and transport mainly due to a decrease in perception of acidity and typical mandarin aroma and accumulation of off-flavors. Aroma profiling analysis of homogenized segments of Or and Mor mandarins conducted by gas chromatography mass spectrometry (GC-MS) revealed that the contents of some aroma volatiles, mainly aldehydes and terpenes, somewhat decreased during storage, whereas contents of other volatiles, especially the ethanol fermentation metabolites ethanol and acetaldehyde, and various ethyl esters which are fatty acid and amino acid catabolism products significantly increased during storage. These biochemical observations were further supported by complimentary genome-wide transcript profiling analysis studies conducted using the Affymetrix Citrus Genome Array, which revealed correlative increases in gene expression patterns related to ethanol fermentation metabolism and lipid and amino acid catabolism. Overall, we propose that simultaneous induction of ethanol fermentation metabolism and lipid and amino acid catabolism, most probably for energy production means, are involved in causing off-flavors in mandarin fruit after harvest. Furthermore, we propose that high levels of ethanol serve as substrates for subsequent downstream esterification reactions with acyl-CoA's derived from fatty acid and amino acid catabolism; a reaction catalyzed by alcohol acyl transferases (AAT's), which results in accumulation of various ethyl esters volatiles imparting undesired off-flavor odors.

S21O02

Traceability of citrus fruit using isotopic and chemical markers

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Sicilian citriculture is currently involved in a marked crisis mainly related to the lack of a commercial strategy along the whole chain of production. This crisis can be overcome focusing on typical citrus productions, which have been awarded PGI (Protected Geographical Indication) status by the European Commission. Thus it comes the need of a system of traceability to verify the authenticity of these productions. The aim of this research was to classify, by a chemical and chemometric approach, Sicilian typical citrus productions (Arancia Rossa di Sicilia and Limone di Siracusa) whose geographical origin was authenticated by samplings made in PGI or not-PGI areas. The joint use of chemical (quality parameters), spectral (NIR spectral patterns) and isotopic (13C/12C, 18O/16O, 2H/1H) markers has revealed that a representative database of each geographical area can be used for the development of a traceability system of such typical productions. Besides, a growth potential for the Sicilian citriculture is the conversion to organic production. Thus, the question of the authenticity of foods labeled as organic becomes an imperative requirement. Our recent researches, aimed at identifying new markers by monitoring the δ15N and other components derived from primary and/or secondary metabolism in citrus fruits from organic and conventional commercial farms and experimental fields, have shown that δ15N analysis may contribute to the differentiation between organic and conventional fruit. Moreover, in case of supply of organic fertilizers in conventional regime, a model of multivariate analysis, including δ15N and other quality parameters (TSS, TA, ascorbic acid, total polyphenols, ORAC units), can contribute to a reliable discrimination between organic and conventional fruit.

S21O03

Fluctuation of limonin and nomilin content in different tissues during fruit development of three sweet orange varieties

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In this study, limonin and nomilin content in different tissues of fruit at different developmental stages of three sweet orange varieties (*Citrus sinensis*), 'Xuegan' 'No 7 Zhongyu Jincheng' and 'Fengqi' navel were

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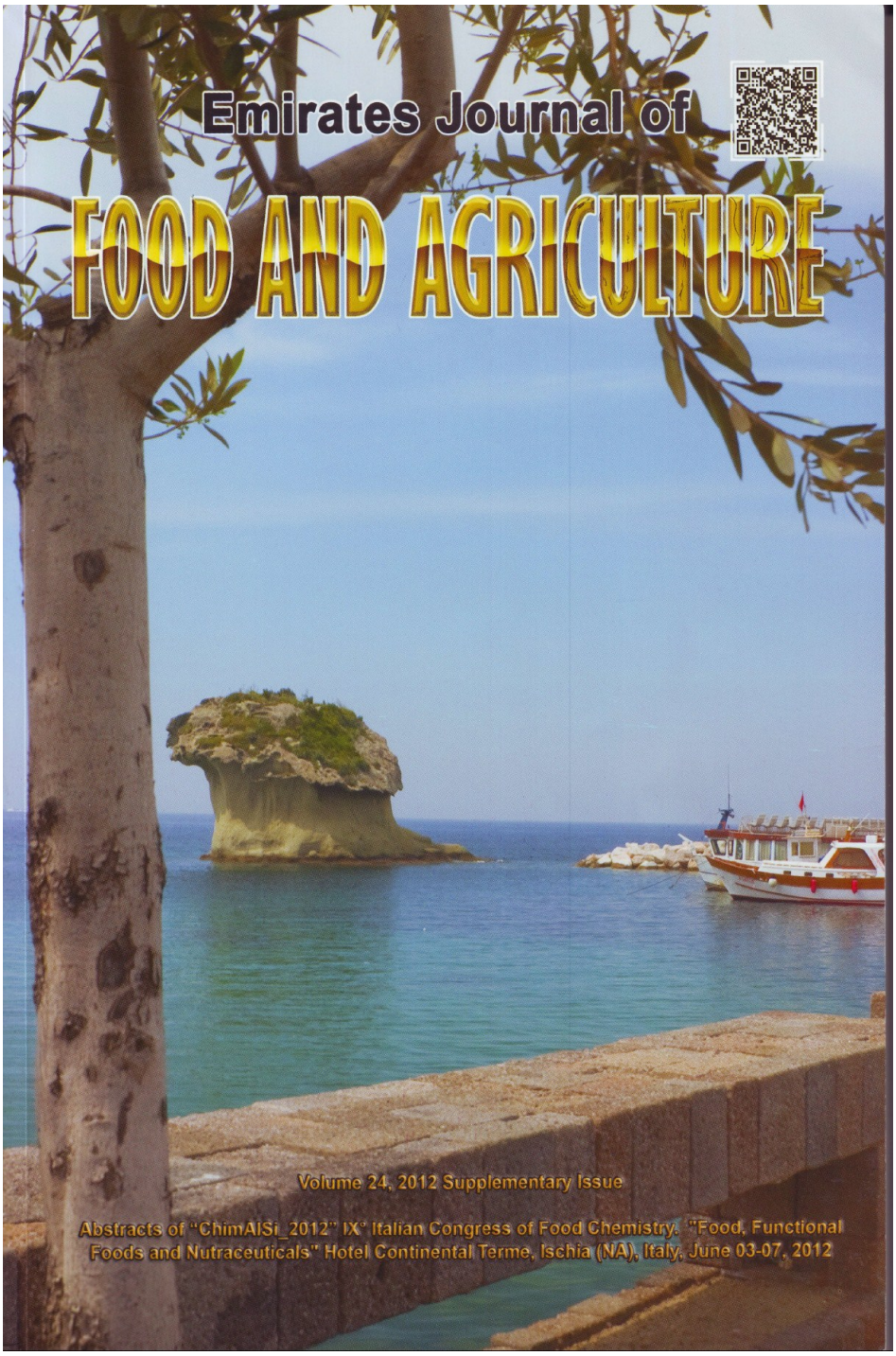
Emirates Journal of
FOOD AND AGRICULTURE
Volume 24, 2012 Supplementary Issue
Abstracts of "ChimAlSi_2012" IX° Italian Congress of Food Chemistry. "Food, Functional Foods and Nutraceuticals" Hotel Continental Terme, Ischia (NA), Italy, June 03-07, 2012

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P-166

Susceptibility to denaturation of caseins in milk samples for improving protein conformational study and their identification

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Abstract
Caseins are phosphoproteins and constitute the major protein component of bovine milk. Caseins occur as micelles in the native form, which are kept together by non-covalent interactions between proteins and by calcium phosphate linkages and appear as a highly stabilized dispersion in milk. In order to optimize the chromatographic resolution for a better identification of individual casein fractions, in this work were analyzed the different effects of denaturing solvents and solutions on the structural conformation of caseins. The caseins were obtained from skim milks by precipitation at pH 4.3, and the proteins was dissolved in: water (solution A); 8 M urea in water/acetonitrile (70:30 v/v) (solution B); 0.3% (v/v) β-mercaptoethanol in water/acetonitrile (70:30 v/v) (solution C); 8 M urea in 165 mM Tris-HCl, 44 mM sodium citrate and 0.3% β-mercaptoethanol (solution D). The chromatographic separation of caseins was performed by reversed-phase high-performance liquid chromatographic (RP-HPLC) using a C4 column and the each casein was identified by MALDI-TOF MS. The best chromatographic separation was achieved by treatment of casein powder with solution D. The CD-spectrum of caseins dissolved in this denaturing solution, showed a significant increase in the α-helix conformation and a decrease in the rate of β-sheet, while random coil conformation remained unchanged. This treatment allowed the separation of different casein sub-fractions and the identification of each component of casein portion.

P-167

Authentication of the geographical origin of Sicilian typical *Citrus* productions

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Abstract
Sicily is characterized by optimal pedoclimatic conditions for the production of citrus fruits, as evidenced by the wide germplasm. Blood oranges (cv. Tarocco, Moro e Sanguinello), which are mainly produced in eastern Sicily and much appreciated in Italian and foreign markets for their organoleptic and nutritional properties, obtained the recognition of the European Union as PGI (Protected Geographical Indication) 'Arancia Rossa di Sicilia' in 1996. Similarly, lemon fruits cv. 'Femminello Siracusano', grown in Siracusa district, has recently been recognized as PGI ('Limone di Siracusa') and has valuable market shares within Italian and foreign markets. However, Sicilian citriculture is currently involved in a marked crisis mainly related to the lack of a concrete commercial strategy along the whole chain of production. This crisis can be overcome focusing on the valorization of typical citrus productions, which have been awarded PGI status by the European Commission, through the implementation of a reliable system to verify the authenticity of these productions and the effective compliance to production regulations. The aim of this research was to classify, by a chemometric approach, Sicilian typical citrus productions whose geographical origin was authenticated by samplings specifically made in PGI or not-PGI areas. Blood orange (cv. 'Tarocco') and lemon (cv. 'Femminello Siracusano') fruits sampled in three years (2010-2012) in PGI and not-PGI areas, have been analyzed respect to the standard quality parameters, the NIR spectral pattern, the trace element profile and the multielement stable isotopic characteristics ($^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{2}H/^{1}H$). The collected data have been subsequently processed by a multivariate statistical approach (PCA, Principal Component Analysis and LDA, Linear Discriminant Analysis) in order to evaluate the feasibility to differentiate PGI and not-PGI productions. In conclusion, the joint use of chemical, spectral and isotopic markers has revealed that a representative database of each geographical area can be used for the development of a traceability system of such typical productions.

FORMATIVE ACTIVITY

Corso di Spettrometria di Massa organizzato dal CRA-OLI Gennaio 2012 ,CRA-OLI, Rende (CS)

Corso di Statistica multivariata organizzato dal CRA-ACM, Marzo 2012, Acireale (CT)

Corso di Cromatografia multidimensionale HPLC-HRGC-MS organizzato da ERRECI S.R.L, Marzo 2011 Hotel Sheraton, Acicastello (CT)

PARTICIPATION IN CONGRESS

Convegno tecnico scientifico "Progettazione, realizzazione e manutenzione di laboratori e di aree sanitarie ai sensi della vigente normativa di igiene e sicurezza" organizzato da CO.PRO.LAB 14 Giugno 2012 a Catania

IX Congresso Italiano di Chimica degli alimenti "ChimALSI" svoltosi a Ischia dal 03 al 07 GIUGNO 2012

Primo Congresso Nazionale della Rete Italiana per la Ricerca in Agricoltura Biologica – RIRAB "L'agricoltura biologica in risposta alle sfide del futuro: il sostegno della ricerca e dell'innovazione" svoltosi dal 07 al 08 Novembre 2011 presso Palazzo Platamone (CT

1st Annual meeting of the Athena Project svoltosi il 13-14 Ottobre 2011 presso il CRA-ACM , Acireale

ALIMED 2011Mediterranean Diet Congress: Quality, Safety and Health, 22-25 Maggio 2011 Orto Botanico, Palermo (PA)

8º Convegno AISTEC "Evoluzione e rilancio della filiera dei cereali: biodiversità, sostenibilità, tecnologia e nutrizione, 11-13 Maggio 2011 Gran Hotel Baia Verde, Acicastello (CT)

Convegno La PAC dopo il 2013 "Il processo di riforma della nuova Politica agricola comune", 4 Marzo 2011 Ciminiere, Catania (CT)

Convegno Internazionale Aromi alimentari nell'Unione Europea "Sviluppi recenti e prospettive future in ambito regolatorio e scientifico", 15 Febbraio 2011 Università degli studi di Milano, Milano (MI)

VIII Congresso Nazionale di Chimica degli Alimenti "Qualità e tipicità degli Alimenti Mediterranei: Alimentazione e Salute", 20-24 Settembre 2010 Hotel Resort Villa Favorita, Marsala (TP)

Convegno "Alimenti Mediterranei e Benessere*",* 05 Giugno 2010 CRA- Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee Acireale (CT)