**Universidad Politécnica de Cartagena. Departamento de
Ingeniería de Alimentos y del Equipamiento Agrícola.
ETSIA**

**RECUPERACIÓN DE VARIEDADES INFRAUTILIZADAS
DE FRUTALES Y DE PLANTAS COMESTIBLES
SILVESTRES PARA SU USO EN LA ALIMENTACIÓN
MODERNA**

**RECOVERY OF UNDERUTILIZED VARIETIES OF FRUIT
TREES AND WILD EDIBLE PLANTS FOR THEIR USE IN
MODERN NUTRITION**

Ana Romojaro Casado

2014

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Universidad Miguel Hernández. Departamento de Biología Aplicada. EPSO.

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PhD. Thesis

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**CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL
POR LA COMISIÓN ACADÉMICA DEL PROGRAMA**

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ALIMENTARIO

INFORMA:

Que la Tesis Doctoral titulada, "Recuperación de variedades infrautilizadas de frutales y de plantas comestibles silvestres para su uso en la alimentación moderna", ha sido realizada, dentro del mencionado programa de doctorado, por D^a Ana Romojaro Casado bajo la dirección y supervisión de la Dra. M^a Teresa Pretel Pretel.

En reunión de la Comisión Académica de fecha 2/05/14, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización del Director de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por la Comisión de Doctorado.

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En Cartagena, a 12 de Mayo de 2014

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Resumen

RESUMEN

En la actualidad, el sector agroalimentario está sufriendo cambios a muchos niveles, tanto en el ámbito de su rentabilidad, como de naturaleza sociológica y ecológica. Es necesario reforzar el paradigma de la diversidad en todos sus componentes, biodiversidad, agrobiodiversidad o diversidad cultural y alimentaria. Ante esta realidad, con la presente tesis se pretende apoyar la recuperación de antiguas especies y variedades vegetales hoy infrautilizadas u olvidadas. Muchas de estas especies presentan altos valores nutricionales, dietéticos o incluso medicinales y podrían utilizarse para el desarrollo de productos innovadores en la gastronomía actual.

En primer lugar, se desarrolló un producto de cuarta gama a partir de variedades infrautilizadas de cítricos. Para ello, se seleccionó una variedad tradicional de naranja, *Citrus sinensis* (L.) Osbeck cv. Cadenera, y se optimizaron las condiciones de pelado enzimático y de conservación más adecuadas para obtener un producto de calidad. Los resultados mostraron que las mejores condiciones para obtener gajos de esta variedad mediante pelado enzimático fueron 1 ml/L de Peelzym II aplicado a 53 kPa durante tres pulsos de dos minutos y un periodo de incubación de 30 minutos con la disolución enzimática a presión atmosférica. Los gajos fueron envasados en dos films de diferente permeabilidad y almacenados durante 10 días a 4°C. El color (a/b) se potenció con el proceso de pelado y se mantuvo en ambos films sin variaciones importantes durante todo el periodo de conservación. Algunos parámetros como la pérdida de peso y la capacidad antioxidante disminuyeron ligeramente después de 10 días de conservación. La flora microbiana se redujo después del pelado enzimático. Tanto la calidad sensorial, como la microbiológica (microflora mesófila, mohos y levaduras y microflora láctica), se mantuvieron en niveles adecuados para el consumo durante 7 días. Se obtuvo por tanto un producto de alta gama a partir de una variedad infrautilizada de naranja.

En segundo lugar, se seleccionaron trece especies de plantas comestibles silvestres (PCS) pertenecientes a once familias botánicas utilizadas tradicionalmente como alimento, y se analizaron diversos parámetros como la actividad antioxidante, el contenido en ácidos orgánicos, la composición mineral y características sensoriales, lo que nos permitió seleccionar aquellas especies de mayor interés nutricional o funcional. *Sanguisorba minor* Scop., *Quercus ballota* Desf. y *Sedum sediforme* Pau. mostraron la mayor actividad antioxidante total (TAA) así como el mayor contenido en fenoles totales. *Asparagus acutifolius* L., *Allium ampeloprasum* L., *Foeniculum vulgare* Mill. y *Malva sylvestris* L. presentaron altos niveles de potasio. *Malva* y *Asparagus* resultaron interesantes debido a su contenido en zinc y *Urtica urens* L. por sus altos niveles de calcio. El análisis sensorial indicó que los frutos de *Q. ballota* podrían considerarse muy dulces y las plantas de *Crithmum maritimum* L. y *Oxalis pes-caprae* L., muy ácidas. Además, los catadores destacaron el sabor salado de *C. maritimum*, *Mesembryanthemum*

nodiflorum L. y *Mesembryanthemum cristalinum* L.; el sabor picante de *A. ampeloprasum* y el aroma de *F. vulgare*. Nuestros resultados indican que un aumento del consumo de las especies estudiadas implicaría beneficios para la salud. Además, debido a sus propiedades sensoriales, podrían utilizarse como nuevos ingredientes para aumentar la variedad en la dieta moderna y en la alta cocina creativa.

Las PCS más adecuadas (frutos de *Rosa canina* L., frutos de *Q. ballota* y hojas y tallos jóvenes de *S. minor*) se adicionaron a matrices de frutas y a aceites vegetales comestibles para diseñar nuevas bebidas enriquecidas con ingredientes bioactivos y/o protegerlas frente a la oxidación. La adición de PCS a zumo de naranja y cremogenado de kiwi no afectó, e incluso mejoró, algunos parámetros de aceptación por parte de los consumidores. La adición de *R. canina* y *S. minor* aumentó el contenido de compuestos fenólicos y la capacidad antioxidante en ambas matrices (zumo de naranja y cremogenado de kiwi) en más de 30 %. Las tres PCS aumentaron la estabilidad oxidativa del aceite de girasol mientras que, en el aceite de oliva, el efecto de estas PCS frente a la disminución de la peroxidación lipídica sólo fue evidente para la concentración más alta. Por lo tanto, estas plantas comestibles silvestres podrían utilizarse para el diseño de nuevas bebidas, combinadas con zumos o cremas vegetales; así como para el enriquecimiento de aceites vegetales comestibles con bajo contenido de antioxidantes naturales, tales como aceite de girasol y evitar o disminuir así el uso de antioxidantes sintéticos.

Los resultados obtenidos en este trabajo a partir de especies vegetales infrautilizadas, se podrán tener en cuenta a la hora de diseñar nuevos productos para la alimentación actual. Asimismo, los resultados de este estudio se podrán utilizar para potenciar la conservación de los recursos genéticos locales, lo que ayudará al mantenimiento de la biodiversidad.

Abstract

ABSTRACT

At present, the agrifood industry is suffering changes both regarding profitability and at sociological and ecologic levels. The reinforcement of the paradigm of diversity in all its components, biodiversity, agrodiversity or cultural and food diversity, is needed. Considering this reality, this thesis tries to support the recovery of old vegetables species and varieties that are now underutilized or forgotten. Many of these species show high nutritional, dietary and even medicinal values, and they could be employed for the development of innovative products in current gastronomy.

Firstly, a fresh-cut product was developed from underutilized varieties of citrus fruits. A traditional variety of orange, *Citrus sinensis* (L.) Osbeck cv. Cadenera, was selected and the most adequate conditions of enzymatic peeling and storage were optimised in order to obtain a quality product. The results showed that the best condition to obtain Cadenera segments by enzymatic peeling was 1 ml/L of Peelzym II applied at 53 kPa with three vacuum pulses of two minutes and a subsequent period of 30 minutes in the enzymatic solution at atmospheric pressure. The segments were packaged in two films with different permeability and stored during 10 days at 4 °C. Colour (a/b) increased with the peeling process and it remained without significant changes during the whole storage period for both films. Some parameters, like the weight loss and the antioxidant capacity, slightly decreased after 10 days of storage. The microbial flora decreased after the enzymatic peeling. Both the sensory and the microbiological quality (mesophilic microflora, moulds and yeasts and lactic microflora) were kept in adequate levels for consumption during 7 days. Therefore, a fresh-cut product was developed from an underutilized variety of orange.

Secondly, thirteen species of wild edible plants (WEP) from eleven botanical families that have traditionally employed as food were selected, and different parameters like the antioxidant activity, the content in organic acids, the mineral composition and the sensory characteristics, were analysed. This allows the selection of the species with a higher nutritional or functional interest. *Sanguisorba minor* Scop., *Quercus ballota* Desf. and *Sedum sediforme* Pau. showed the highest total antioxidant activity (TAA) and the highest content in total phenols. *Asparagus acutifolius* L., *Allium ampeloprasum* L., *Foeniculum vulgare* Mill. and *Malva sylvestris* L. showed high levels of potassium. *Malva* and *Asparagus* were interesting due to their high content in zinc and, *Urtica urens* L. presented high levels of calcium. The sensory analysis indicated that fruits from *Q. ballota* could be considered very sweet and plants of *Crithmum maritimum* L. and *Oxalis pes-caprae* L. were very acidic. Moreover, testers highlighted the salty taste of *C. maritimum*, *Mesembryanthemum nodiflorum* L. and *Mesembryanthemum cristalinum* L., the spicy taste of *A. ampeloprasum*, and the aroma of *F. vulgare*. Our results indicate that an increased consumption of the investigated plant species could provide health benefits.

Moreover, due to their sensorial properties, they could be used as new ingredients to improve the diversity in modern diet and highly creative cuisine.

The most adequate WEP (fruits from *Rosa canina* L., fruits from *Q. ballota* and leaves and young stems of *S. minor*) were added to matrices of fruits and to edible plant oils in order to design new drinks enriched with bioactive ingredients and/or to protect against oxidation. The addition of WEP to orange juice and kiwifruit cremogenate did not affect, and even improved, some parameters of acceptance by consumers. The addition of *R. canina* and *S. minor* increased the content of phenolic compounds and the antioxidant capacity in both matrices (orange juice and kiwi cremogenate) in more than 30 %. The three WEP increased the oxidative stability of sunflower oil while the effect of these WEP against the decrease of the lipid peroxidation in olive oil was evident just for the highest concentration. Therefore, these wild edible plants could be employed for designing new drinks combined with juices or plant creams, as well as for enriching edible plant oils with low content in natural antioxidants, like sunflower oil, and for avoiding or reducing the use of synthetic antioxidants.

The results obtained in this work from underutilized plant species could be considered when designing new products for current diets. Likewise, they could be also used for promoting the conservation of local genetic resources that can keep biodiversity.

Introduction

1. BIODIVERSITY AND PLANT GENETIC RESOURCES

The term biodiversity (from Greek *Bio-*, life, and Latin *diversitas*, *-ātis*, variety), also called biological diversity, refers to the variety of living beings on Earth that results from thousand million years of evolution following natural processes, and from the increasing influence of human activities (Izco et al., 2004). In the monograph of the United Nations Environment Programme (UNEP), biodiversity is defined as the total diversity and variability of living organisms and the systems of which they are part. This includes the variation of systems and organisms and the variation among them in different levels, from the bioregions to particular habitats, and in any organisation level, including those under species level.

The intrinsic value of biodiversity comes from the fact that it is the result and the expression of a very ancient natural historic process. Just for this reason, biological diversity has the inalienable right to continue its existence. Humans and their culture, as a product and part of this diversity, must protect and respect it, since it is a guarantee of both well-being and the balance of the biosphere. Finally, from our human condition (anthropocentric point of view), biodiversity is also a natural capital. The use and benefit of biodiversity has contributed in many different ways to the development of human culture, and it represents a potential source for future needs at ecological, economic and scientific levels.

In 2010, the Strategic Plan for Biodiversity 2011-2020 was set in motion, a framework for action for all countries to safeguard biodiversity and its benefits for people. As part of the Strategic Plan, 20 targets, known as Aichi Biodiversity Targets, were established. This Strategic Plan tries to take effective and urgent measures for stopping the loss of biodiversity in order to guarantee that, in 2020, the ecosystems will be resilient and they will continue providing essential services that can ensure the variety of life in planet and contribute to human well-being and poverty eradication.

One of these targets is related to the conservation of plant species. It also sets a relatively low number of cultivated plants from which we depend in order to obtain food and fibres. There are many other wild plants with a great cultural and economic significance since they provide humans with food, medicines, fuel, dress and shelter. Many of them are endangered plant species. They are threatened by changes in their habitats, overexploitation, invasive exotic species, pollution and climatic change. The disappearance of these significant components of biodiversity is one of the main risks that humans must face.

1.1. Historical development of the conservation of plant genetic resources

During the twenties and the thirties of 20th century, the first systematic study about variation of cultivated plants was carried out by identifying the geographic areas with maximum genetic diversity of these species, areas that were named Centres of Diversity of Cultivated Plants of Vavilov: Central America and Mexico, Andean Area, Mediterranean Area, Central Asia, Brazil and Paraguay, Near East, Chile, China, Ethiopia, India and Indo-Malaysia. These centres of diversity were employed as genetic "treasure maps" after II World War in many different European and American projects of conservation of plant genetic resources (Pistorius, 1997). Vavilov and his team made expeditions in more than 50 countries and they collect 50 000 samples of seeds of wheat, rye, oats, peas, lentils, chickpeas and corn. This way, they laid the foundations for the creation of modern germplasm banks in USSR. Vavilov was ahead of his time because the coordinated actions of systematic rescue of genetic variability did not start until some decades after (Esquinas-Alcázar, 1981; 1993).

During the forties of the past century, other scientists, with a forward-looking approach, posed the problem of the genetic erosion (loss of diversity) in agriculture. However, until the sixties, the ideas about the need of collecting and storing the threatened plant genetic resources of crops in germplasm banks were not totally accepted. A significant milestone in the history of agricultural biodiversity conservation was the 1967 FAO/IBP Technical Conference on the "Exploration, Utilization and Conservation of Plant Genetic Resources", arranged by the International Biological Programme (IBP) and the Food and Agriculture Organization of the United Nations (FAO). This was one of the first occasions on which a group of experts met for defining a global strategy about the conservation of plant genetic resources. This group of experts was later known as the "Panel of Experts on Plant Genetic Resources". At the end of the sixties of 20th century, and despite the fact that the acknowledgment of environmental problems was increasing, the concern about the genetic erosion was mainly due to agronomists (Pistorius, 1997).

However, this concern about genetic erosion had not attracted a majority attention from the public. It was at the beginning of the seventies when two serious consequences of the genetic erosion aroused an unexpected attention and acknowledgement of the problem. In 1970, there was a disastrous attack of *Helminthosporium maydis* that drastically reduced the corn crop in the United States and destroyed more than 50 percent of the cornfields in the South of the country. The cause of the disaster was blamed on the genetic uniformity of corn, mainly due to the fact that all cultivated hybrids shared the same cytoplasm. This same year, a catastrophic epidemic of coffee rust caused significant losses in Brazil and an increase of the price of coffee in world markets. These two cases,

together with others that were later known, were compiled in a report published by the USA National Academy of Sciences (NAS) on the “Genetic Vulnerability of Major Crops”.

In 1974, the Consultative Group on International Agricultural Research (CGIAR) created the International Board for Plant Genetic Resources (IBPGR). This organisation, working under the auspices of FAO, has the explicit mandate to collect the threatened plant genetic resources and to facilitate their long-term storage using an internationally coordinated network. It was originally established in 1983 in the FAO conference as the Commission on Plant Genetic Resources in charge of those issues related to plant genetic resources. In 1995, its competences were broadened in order to include all components of agrobiodiversity for food and agriculture. Therefore, the name changed to Commission on Genetic Resources for Food and Agriculture (CRGAA). Nowadays, 160 countries and the European Union are members of the Commission and it is open to all Associate Members of FAO, upon request. The CRGAA deals with policy, sectorial and cross-sectorial matters related to the conservation and use of genetic resources of relevance to food and agriculture. It also develops and supervises the global strategy for the management of genetic resources as well as facilitates and oversees cooperation between FAO and other competent bodies, particularly the Conference of Parties to the Convention of Biological Diversity (CBD), the United Nations Commission on Sustainable Development (CSD) and the International Plant Genetic Resources Institute (IPGRI, today Biodiversity International).

During the International Technical Conference on Plant Genetic Resources, held in Leipzig in June 1996, the Global Plan of Action (GPA) for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture of FAO was adopted, and in 2011 the second GPA, an update of the previous one, was approved. The second GPA includes a set of priority activities that directly address the new developments, opportunities and challenges facing plant conservation and use in the 21st century. These include new policies and international agreements that affect conservation, use and exchange of genetic resources, shift in food production trends, changing roles of public and private sector in crop improvement and delivery systems, advances in biotechnology, genomics and information technologies, new products derived from agriculture, impact of new pests, climate change and rapid urbanization on plant genetic erosion and vulnerability. The priority activities of the Second GPA addresses these developments to ensure that plant genetic resources for food and agriculture (PGRFA) continue to be available for current and future use for food security and sustainable agriculture.

1.2. Plant Genetic Resources For Food and Agriculture (PGRFA)

Both the CBD and the GPA agree that the conservation and use of the plant genetic diversity are essential for satisfying the needs of the future development of the world.

The PGRFA are the biological base of the agricultural production and the world food security because they allow adapting crops and varieties to changeable conditions, pests, diseases and abiotic stresses. These resources are the most significant raw material for the farmers and plant breeders to contribute to the achievement of a sustainable agricultural production.

Esquinas-Alcázar (2007) highlights the value of traditional varieties and of wild relatives of cultivated plants for plant breeding and agricultural development. Wild plants could have acquired many desirable characteristics as a consequence of their long exposure to natural selection and, therefore, their contribution to plant breeding can be very useful. An exceptional example is the genus *Lycopersicon*, in which many wild species can be crossed with cultivated tomato *Lycopersicon esculentum* Mill. and that have been successfully employed as donors of fungus-resistant genes (*Lycopersicon hirsutum* Dunal and *Lycopersicon peruvianum* (L.) Mill), insect-resistant genes (*Lycopersicon hirsutum*), genes for improving quality (*Lycopersicon chmielewskii* C.M. Rick, Kesicki, Fobes and M.Holle), and genes for adaptation to adverse environments (*Lycopersicon cheesmaniae* Riley). Similar examples could be mentioned for most crops. These examples show that the genetic material which was sometimes considered not valuable was essential for crop improving. The concept of “utility” is relative and can be different according to the needs and the available information.

The world population is estimated to double or treble before its stabilization at the end of the 21st century, and it is expected that in 2050 more than 70 % of world population will be urban population (compared to the present proportion of approx. 50 %). These facts will create strong pressures on the environment and will need a significant increase of the production of goods, both food and non-food ones. The main causes of genetic erosion mentioned in FAO reports of 2006 and 2011 are the displacement of local varieties, deforestation, overexploitation of species, demographic pressure, excessive grazing, environmental degradation, inadequate legislation, change of the agricultural system, effect of diseases, pests and weeds, reduction of fallow areas and internal conflicts. The conservation, the sustainable use and the fair and equitable distribution of the benefits from the use of plant genetic resources are now an international concern.

The world is facing an increasing food insecurity that can be observed in the price volatility of food products. The climate change, the increase of housing development, the need for a higher sustainability of agriculture, and the need to safeguard the plant genetic diversity require more attention to conservation and utilization of plant genetic resources for food and agriculture. In practically the whole developed world, the supply of most food products is in the hands of industrial production systems boosted by the strong demand by the consumers of cheap foods with uniform and foreseeable quality. The crop varieties are selected for satisfying the needs of these systems and of strict market

regulations, usually in one-crop production systems, but also for facing biotic resistances, nutritional quality and yield stability. These novelties have reinforced the trend towards the decrease of the specific genetic diversity of farmers' fields.

However, the developing world still produces a significant part of the foods with few or any chemical consumables, and there are million small-scale farmers in the whole world that depend on the locally available PGRFA for their subsistence and well-being. At the same time, there are new opportunities that can improve the management of those resources, like the powerful communication and information technologies as well as the significant advances in biotechnology and the development of bio based products derived from agriculture. The new technologies have significantly increased the amount of information about the extent and nature of the genetic erosion and vulnerability of PGRFA. This increase of information and technological resources has boosted the international commitment to research and development activities on agriculture and food.

Target 13 of the "Aichi Biodiversity Targets" is essential in relation to genetic diversity: "By 2020, the genetic diversity of cultivated plants and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity." Some of the other targets are also related to conservation and sustainable use of plant genetic resources. The Second GPA has the aim to significantly contribute to the achievement of those targets, and new challenges and opportunities are faced through 18 priority activities. Among these actions, the promotion of development and commercialization of all varieties, mainly farmers' varieties/landraces and underutilized species is included.

Natural ecosystems are home to important PGRFA, including rare, endemic and threatened Crop Wild Relatives Species (CWR) and edible wild plants. The best way of conservation of CWR and wild plants is *in situ*, where they can evolve under natural conditions. Unique and particularly diverse populations of these species must be protected *in situ* when they are under threat. However, most of the world's national parks and other protected areas were established with little attention to conservation of genetic diversity of plants, especially of CWR and edible wild plants.

One of the aims of the Second GPA is the sustainable use of genetic resources of CWR and wild edible plants and their conservation in protected areas and on lands not explicitly listed as protected areas. Another aim is the promotion of planning and management activities in important *in situ* conservation areas for CWR and edible wild plants, as well as to assess the threats to priority CWR and wild edible plants and their conservation status, and to develop management plans for their protection *in situ*. The rest of aims include to improve knowledge of the uses of wild plants as source of income

and food; to obtain a better understanding of the contribution of CWR and wild plants to local economy, food security and environmental health; to improve management and planning and promote complementarity between conservation and sustainable use in parks and protected areas by, among other things, increasing the participation of indigenous and local communities in ensuring the active conservation of CWR and wild edible plants genetic diversity; and to establish a better communication and coordination among the different bodies engaged in *in situ* conservation and land-use management at national and regional levels, especially between the agriculture and environment sectors.

On the other side, in commercial production systems, a few significant crops cover a large proportion of global needs. However, many species, including varieties used by farmers and indigenous and local communities, are used to satisfy the local demand of food, fibres and medicines. This diversity of species and varieties is being increasingly replaced by a certain level of uniformity in the agricultural market since the varieties are bred to meet the needs of production, industrial processing and demanding market standards.

Therefore, one of the aims of the Second GPA is the documentation of local and traditional knowledge and the identification of all varieties, especially traditional and underutilized species. Likewise, this GPA tries to develop post-harvest processing methods for improving marketing possibilities of traditional and underutilized species, and to develop marketing strategies and brand creation for these underutilized species and varieties. Therefore, research is needed to identify the marketing possibilities of these species and varieties. In this sense, Scherr et al. (2008) assessed the potentials of and limitations to conservation of biodiversity in productive agricultural landscapes and reached a conclusion that the feasible conservation of wild biodiversity in agricultural landscapes requires more research, policies coordination and strategic support to agricultural communities and conservationists.

2. UNDERUTILIZED VARIETIES OF FRUIT TREES AND WILD EDIBLE PLANTS

Two moments in history have represented significant changes in human diet: the appearance of agriculture and the beginning of the industrial era (Tardío et al., 2011). In 1985, Eaton and Konner posed the hypothesis of the evolutionary discordance that proposes that these changes in the diet are responsible for many health problems suffered by the present western world because our organisms have not yet incorporated the necessary genetic changes to correctly metabolize those foods. Among those health problems, typical of our society, chronic diseases like diabetes, cardiovascular diseases,

hypertension, obesity and some types of cancer have been mentioned. Since then, many other scientists have carried out epidemiological and clinic studies that seem to confirm, at least, a great part of this theory (Cordain et al., 2005; Frassetto et al., 2009; Jonsson et al., 2006; Jonsson et al., 2009; Simopoulos, 2003).

According to Konner and Eaton (2010), the human genetic constitution has changed relatively little since the appearance of our present species, *Homo sapiens*, around 40 000 years ago. Due to the low rates of spontaneous mutation of chromosomal DNA since the beginning of agriculture, around 10 000 years ago, time has allowed only a few changes in the human genome. During this whole period, our ancestors and a few present cultures were hunters-gatherers and consumed wild foods of both animal (lean meat and bone marrow, fish, shellfish, eggs) and plant (vegetables, berries, dried fruits, roots) origin. However, this diet did not include cereal grains or legumes or dairy products that, together with sugar, salt and saturated fats, have become basic foods of our current diet. According to different authors (Arsuaga and Martínez, 1998; Carbonell and Pastó, 2005; Konner and Eaton, 2010), although the introduction of meat in diet was crucial for human evolution (Arsuaga and Martínez, 1998; Carbonell and Pastó, 2005), vegetables still were a significant part of it.

Nowadays, around 50 % of world requirements of proteins and calories are obtained from just three species: corn, wheat and rice (FAO, 1996, 2011), and only 150 crops are commercialized at a global significant scale. On the other side, ethnobotanical studies point out that there are more than 7 000 plant species cultivated or collected from the wild (Padulosi et al., 2002; Wilson, 1992). These species are an excellent alternative for diversifying and increasing the number of foods in a diet, and for obtaining nutritional benefits subjugated by an industrial and universal feeding regime that offers more and more packaged products (FAO, 2011). Wild foods are still interesting due to different reasons. On the first place, nutrition and health reasons; wild edible plants are rich in vitamins and minerals, and sometimes they are consumed due to their restorative value more than to their nutritional value. Some species are a greater source of nutrients than many cultivated plants and, sometimes, they also have medicinal properties. Another reason could be that the great variety of traditional cultivations and wild plants, cooked in different ways, are a source of new tastes that could extend the reduced range of vegetables currently consumed (Tardío et al., 2002).

As a consequence of this impoverishment, we find great agricultural areas dedicated to the cultivation of just one species, and a higher and higher dependence on chemical fertilizers, insecticides, fungicides and herbicides for obtaining satisfactory crops. There is a constant threat to develop new diseases or resistance of insects to chemicals that can cause serious losses in great concentrations of just one crop. Global

climate changes can also cause significant changes in agriculture. Therefore, a higher crop diversification is urgently needed (FAO, 2011).

Local and traditional cultivations (with their ecotypes and local varieties) and wild species represent richness for agrobiodiversity and they have a great potential for improving income, food security and nutrition, and for fighting hunger caused by micronutrient (vitamins and minerals) deficiencies. They are also closely linked to the cultural inheritance of their places of origin. They are collected from the wild or they are produced under production systems in localized areas for traditional uses, with little or any external supply. They tend to be adapted to specific agro-ecological niches and marginal lands.

These species do not receive special attention by researchers, farmers, politicians, donors, technological firms or consumers, although they can be very nutritious and they have medicinal properties and many other uses. In fact, it has been observed that, in general terms, wild plants tend to be richer in micronutrients and bioactive secondary metabolites than their cultivated relatives. Those secondary metabolites are produced in adaptation to the local conditions of the environment (Heinrich et al., 2005; 2010).

2.1. Wild edible or traditionally harvested plants.

Although the use of wild edible plants decreased in favour of cultivated ones, it was kept in rural areas of many agricultural cultures because they have always been a significant resource in times of seasonal food shortages or famine periods due to bad crops or wars (Benítez, 2009; Mesa, 1996; Pardo de Santayana et al., 2005; Tardío et al., 2005; Bharucha and Pretty, 2010). The continuous use of a great number of species was also a way to keep the knowledge about their edibility, an essential factor in food shortages periods. For instance, thanks to this knowledge and to the use of wild edible plants, the inhabitants of the eastern area of the Kalahari Desert, Botswana, did not starved during long periods of draught (Grivetti, 2006), or the population of Sarajevo could survive the siege they suffered during the recent Balkan wars.

The quantity and the relative use of wild plants vary depending on the countries and, even, the regions. For example, studies carried out by Kuhnlein (2009) show that in Amazonian Awajun and the West African Igbo, 90 % of foods consumed by adults come from local resources, while in Maasai, Pohnpei, Ingano and Bhil; traditional food plant species represent a low percentage of the diet because they have been replaced by white refined wheat or rice flour. In Bangladesh, a study about the dietary intake of 50 families in ten villages showed that non-cultivated local foods were greatly consumed by the poorest families (Mazhar et al., 2007). On the other side, a study on the intake of wild vegetables in the Nanda Devi Biosphere Reserve, in the Himalaya region in India (Misra et

al., 2008), showed that local population uses 21 species belonging to 14 genus, a lower quantity than that consumed in Western Ghats in India and in parts of Africa. The authors suggest that these results point out the erosion of the knowledge about the use of wild plants due to changes in social values and to the lack of implication of younger generations in harvesting and nature processing (Heywood, 2011).

For a long time, a great variety of non-cultivated plants have been employed in the diet of the Mediterranean region (Pardo de Santayana et al., 2005). Therefore, they are also a valuable resource in countries like France, Greece, Italy, Spain and Turkey (Rivera and Obón, 2007; Heywood, 2009, 2011; Egea et al., 2010). The collection of wild plants for food use is a common practice in Greece, especially in Crete and other islands like Cyprus, Sicily and Sardinia. Wild plant gathering for food and other needs is an ongoing tradition and a common practice throughout Anatolian Turkey, and more than 40 wild food species are regularly harvested (Ertug, 2004). On the other side, Tardío et al. (2011) have registered 483 vascular plants, that is, near 6.4 % of the Spanish flora has been used in diet in the different usage categories under consideration (Morales et al., 2011). It must be understood that there is a close relation between the cultural and the biological diversities and that a new social, cultural or economic value must be given to local resources in order to maintain traditions for future generations (Rivera et al., 2006a,b). Moreover, considering the acknowledgement of Mediterranean diet as World Cultural Heritage by UNESCO (2010) and its contribution to health and general welfare, Bach-Faig et al. (2011) proposes that both the adoption of a healthy life style and the protection of cultural elements must be taken into account in order to obtain the benefits from the Mediterranean diet.

The families to which most wild food species belong are similar in the different areas: *Compositae*, *Rosaceae* and *Umbelliferae*, followed by *Boraginaceae*, *Liliaceae*, *Cruciferae* and *Caryophyllaceae*, *Chenopodiaceae*, *Polygonaceae* and *Poaceae*. However, the fact that a family presents a low diversity of edible species is not related to the dietary relevance of this family. For example, the *Caryophyllaceae* family has a relatively low number of species, but bladder campion (*Silene vulgaris* (Moench) Garcke) belongs to this family, and it is one of the most consumed wild plants (Rivera et al., 2005; Pretel et al., 2014). Different studies document traditional uses of wild edible fruits in the Mediterranean region (Fajardo et al., 2007; Rivera et al., 2006a; Egea et al., 2010; Tardío, 2011; Morales et al., 2011).

Among traditionally consumed wild fruits, blackberries (mainly *Rubus ulmifolius* Schott), blackthorn (*Prunus spinosa* L.), strawberry madrone (*Arbutus unedo* L.), acorn (*Quercus ilex* L.) and hawthorn (*Crataegus monogyna* Jacq.) can be highlighted, although the harvest of some of them has now been abandoned (Morales et al., 2011). **Figure 1** shows some examples of fruits that have been traditionally harvested in Spain.

Blackberries are one of the sweetest and tastiest wild fruits, and they can be consumed fresh or in marmalades and liquors (Fajardo et al., 2007). The recipe of blackberry liquor varies from one place to another, although it is usually prepared by macerating fruits in a mixture of spirit and anise with some grains of coffee and cinnamon sticks. The peeled tender shoots are also eaten raw (Rivera et al., 2006a). Although the edible part of the dog rose (*Rosa canina* L.) is very small (only the thin mesocarp), they have an acid and pleasant taste that reminds that of sweet tomato. Fruits of *Rosa canina* are also consumed in infusion for treating colds. Fruits of *Quercus ballota* Desf. have been traditionally used in Spain in casseroles or desserts (there is a great variety with sweet fruits) due to their high content in carbohydrates (Rivera et al., 2006a; Pereira and Garcia, 2002). The use of acorns in human diet has been documented since the end of 19th century in Serbia (Pelagic, 1893), with recommendations about their application and beneficial action. The azarole hawthorns (*Crataegus azarolus* L.) are small rough apples, consumed when they are over-ripened, with a pleasant taste that reminds that of roasted apples. These fruits were eaten during winter and as astringent. Common hawthorn (*Crataegus monogyna*) is consumed raw or employed in marmalades or liquors. Their flowers and leaves are used in popular medicine for treating blood circulation problems. The infusion of flowers of *C. monogyna* is employed for treating colds and as a tranquilizer. Blackthorn (*Prunus spinosa*) is used for spirits that macerate during two or three months.

Among the wild plants that have been traditionally employed in many Spanish regions, watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek), bladder campion (*Silene vulgaris*), wild asparagus (*Asparagus acutifolius* L.), chicory (*Cichorium intybus* L.), wild leek (*Allium ampeloprasum* L.), garden sorrel (*Rumex acetosa* L.) and golden thistles (*Scolymus hispanicus* L.), can be mentioned (Tardío, 2011). Only some of them, like wild asparagus, bladder campion, golden thistles or blinks (*Montia Fontana* L.), are still harvested. Most wild vegetables are widely spread weeds (Tardío, 2010), although some endemisms can be found. Some studies (Tardío et al., 2002; Pretel et al., 2014) register food uses of these vegetables. Chard is widely spread in the peninsula and they are usually cooked with eggs or ham (Tardío et al., 2002). In the province of Alicante (Spain), chards (*Beta vulgaris* L. and *Beta maritima* L.) are consumed boiled together with other vegetables like green beans, onions and potatoes in a dish called *bullit*, a traditional recipe of this Mediterranean region. Asparagus (*Asparagus acutifolius*) and fennel (*Foeniculum vulgare* Mill.) are species employed in recipes with eggs. Fennel is also used as a spice and it is widely spread in the Iberian Peninsula. In Alicante, their tenderest shoots and stems are used in omelettes and pickles, together with pale stonecress (*Sedum sediforme* Pau., slender sow thistle (*Sonchus tenerrimus* L.), caterpillar plant (*Scorpiurus sulcatus* L.) and olives. In many other places of the province of Alicante, it is also known as an aroma agent of wineskins or an organoleptic corrector in the production of cold meats, and it is even consumed raw by chewing or sucking fennel pieces or in sandwiches

(Mulet, 1991; Tardío et al., 2002). Salad burnet (*Sanguisorba minor* Scop.) is a plant in the family *Rosaceae*, a family providing many plants with medicinal uses, mainly in traditional medicine, and it has been traditionally consumed in salads in some areas of Europe (Tardío et al., 2006). In Spain, this species is better known due to its medicinal uses than to the culinary ones, while in Netherlands, Italy or France it is usually eaten in salads (Mulet, 1991). Plants in the family *Asteraceae*, like common sowthistle (*Sonchus oleraceus* L.) and common chicory (*Cichorium intybus*), are consumed fresh, particularly in salads together with other vegetables and a dressing. These species have been traditionally used in rural areas for farm consumption without entering the usual food commercialization channels.

It must be highlighted, therefore, that the Mediterranean diet included, until very recently, different species of wild, semicultivated or cultivated plants that are beneficial to achieve a healthy and long life. These species into disuse have been kept outside those studies carried out in order to know food composition regarding the properties or attributes that have recently aroused interest to dietary intake, like antioxidant activity, content of antioxidant elements, etc. Therefore, we must become aware of the importance of the traditional use of wild or cultivated plants with positive effects on health.

On the other side, due to the advances and innovations in gastronomy, some of those species are again employed and grown. However, those typical recipes with these wild edible species as ingredients are exclusive part of the popular culture. They are usually employed in sauces and salads, but they could be also used as accompaniments, in creams, soups or as part of a vegetables mixture (Pochettino, 2005; Łukasz et al., 2012). Wild edible plants may have a great potential as a source of unusual colours and flavours, bioactive compounds and also as sources of dietary supplements or functional foods (Salvatore et al., 2005; Ranfa et al., 2013).

Moreover, many of these plants could be a source of genetic interest for the development of new products (Ruiz-Rodríguez et al., 2011; Egea et al., 2010; Sánchez-Mata et al., 2012). However, for these wild plants to become an alternative source for the food industry, broader knowledge about their physicochemical, antioxidant, nutritional and toxicological properties is necessary (Flyman and Afolayan, 2006; Łukasz et al., 2012). These studies could be utilised by gastronomic companies to learn about the agro-industrial potential and to promote their exploitation as additives or natural ingredients in the highly creative cuisine.

Figure 1: Wild edible species consumed in Spanish regions: *Sonchus tenerrimus* L. (a); *Sonchus oleraceus* L. (b); *Sedum sediforme* Pau. (c); *Sanguisorba minor* Scop. (d); *Rosa canina* L. (e); *Quercus ballota* Desf. (f).

2.2. Underutilized varieties of fruit trees

The great biodiversity that has characterized until recently the orchards of the Spanish Southeast region is now suffering a significant backward step because, due to many agronomic and economic factors, there is a mass replacement of some varieties and the abandonment of the cultivation of others (Rivera et al., 1998; Pretel et al., 2001). However, some traditional varieties of orange that have been grown for many years still survive in orchards of the Spanish Southeast region; they are well adapted to the Mediterranean climate and they present excellent quality and nutritional value (Pretel et al., 2004). The changes recently suffered by irrigated farming in the different meadows have implied a mass replacement of varieties from some species and the abandonment of the cultivation of others. Therefore, the agronomic and commercial conditions have brought about the abandonment of the cultivation of many citrus fruits varieties that were introduced and adapted to the Spanish Southeast many years ago. This has also caused a landscape impoverishment of the reserve of agricultural resources and of the biodiversity that the orchards of this environment represented. In the present society, the search for possible solutions to the loss of this genetic diversity requires new commercial outlets for these products, paying special attention to current consumers' demands because, in the last years, significant changes in societies with high economic development are opening new perspectives in the food sector. This way, new foods will appear in the market in which consumers will appreciate, among other attributes, the beneficial effects for health and the natural origin.

Among the fruit trees that have been cultivated in the Spanish Southeast, only a few of them can be considered autochthonous since most of them have arrived and adapted to the climate and the local soil in different historic periods (citrus trees, stone fruit trees, etc.) and even in the prehistory (almond trees, pomegranate, common walnut, etc.). However, the continuous cultivation of some of these species could cause the appearance of local forms that can be considered autochthonous. For example, in the Spanish Southeast there are different local varieties of plum that are no more cultivated due to the appearance in the market of more commercially interesting varieties (Egea and Egea, 2010). The condition of centre of genetic diversity of the Spanish Southeast crops reaches global significance regarding the genus *Citrus* (Pretel et al., 2001).

The protection of this diversity has, to a great extent, exclusively depended on the farmers, and it is now threatened by the disappearance of the farmers and their crops due to the abandonment or to the replacement by more productive and bigger varieties that can be better stored. It must be mentioned that only two or three specimens of some cultivarities (groups defined by morphological, physiological and agronomic characters that can present a certain variety inside) are found in the area and, in some cases, in the world (Rivera and Obón, 2007). The conservation of the inventoried genetic diversity is quite difficult if drastic

and immediate measures are not taken. As it has been already mentioned, the reasons for protecting biodiversity in different cultivations and the problems they are affected by have been discussed in international forums from the scientific and the socioeconomic points of view. Some particular problems have been exposed in these forums. Regarding the conservation of citrus fruits in the family *Rutaceae*, the conservation of Asian species has been considered a priority. But it has not been considered that none of the significant cultivations comes directly from them because they have been mostly originated in the Mediterranean area by complex hybridisations. The real immediate ancestors of cultivations are in danger of extinction.

The family of *Rutaceae* includes 150 genus and 1000 species, most of them with a tropical and subtropical distribution. The subfamily *Aurontioideae* presents a higher economic interest because it includes cultivated citrus trees, most of them included in the genus *Citrus*. According to Barrett and Rhodes (1976) only three real species with sexual reproduction can be found within cultivated citrus trees: *Citrus medica* L., *Citrus grandis* (L.) Osbeck and *C. reticulata* Blanco, while the rest (*Citrus aurantifolia* (Christm.) Swingle, *Citrus limon* (L.) Osbeck, *Citrus paradisi* Macfad. or *Citrus sinensis* (L.) Osbeck, are biotypes with a possible hybrid origin, apomictically programmed. Each one of these “species” collectively constitutes a clone. Other authors, like Tanaka (1929) and his followers, split up the genus *Citrus*. too much (Ortiz, 1985; Calabrese, 1973). The systematics of the genus *Citrus*. has been considered from different points of view and the proposal of Rivera *et al.* (1998) will be adopted. These authors take an intermediate position and they accept as real species those taxa more or less stable and relatively little complex in their hybrid origin, while those derived from their crossbreeding are considered hybrids. The ornamental use of citrus trees have contributed to the conservation of useful varieties but, incidentally, with no agronomic interest (Pina, 1989). However, some species with agronomic interest are not cultivated anymore because they have been replaced by more productive varieties during the last years. At present, the society demands a diversity of products and qualities; therefore, the adaptation of new post-harvest technologies to traditional varieties of citrus trees would be an incentive for increasing the commercial interest of these products that can contribute to the conservation of our plant heritage (Pretel *et al.*, 2001).

In the Spanish Southeast some varieties of orange (*Citrus sinensis*) tend to disappear (**Figure 2**), although they have been traditionally consumed as dessert and they have ideal morphological and organoleptic characteristics for the *consumer* (Pretel *et al.*, 2004). According to Kimball (1999) and Rivera *et al.* (1998), the traditional varieties of orange fruit (*Citrus sinensis*) have been classified into five categories Common Group, which are referred to as white or blond oranges (for example, Blanca, Cadenera, Capuchina, Común, Común de Orihuela, Macetera, Salustiana and Verna varieties); Navel Group, which are characterized by a rudimentary secondary fruit in the apex of the primary fruit that resembles a navel (like

Guasi, Navelina, Newhall, and Thompson varieties); Blood Group, which are characterized by their reddish pigmentation, which results primarily from the presence of anthocyanins in the flesh (for example, Condesa and Sanguina Rosada varieties); Acidless Group, also called sugar oranges, characterized by their low or nonexistent acid levels (Grano de Oro and Imperial varieties) and Mollar Group, which have a thick and soft skin (Mollar variety). This orange fruit varieties, differed significantly in fruit weight, juice content and albedo and carpelar membrane weight (Pretel et al., 2004). No correlations were found between juice content and albedo weight, juice content and carpelar membrane weight nor albedo and carpelar membrane weight, showing that the relative proportion of each tissue is different in each variety and independent of fruit size (Pretel et al., 2004).

Figure 2: Traditional varieties of orange (*Citrus sinensis* (L.) Osbeck) in Spanish. Common Group (Blanca-a- and Cadenera -b-); Navel Group (Navelina-c- and Thompson-d-); Blood Group (Condesa and Sanguina Rosada varieties); Acidless Group, (Grano de Oro-g- and Imperial -h-).

3. NUTRIENTS AND ANTIOXIDANTS IN FRUITS AND VEGETABLES

There is not an inherent incompatibility between conservation and utilization of plant genetic resources. In fact, it is essential to guarantee that these activities are totally complementary. Toledo and Burlingame (2006) state that the initiatives combining nutrition and biodiversity are the base for the achievement of the FAO objectives. The composition of fruits provides an essential link for biodiversity and nutrition.

The nutritional interest of many wild plants and their positive influence in health have been stated by many scientists (Flyman and Afolayan, 2006; Grivetti and Ogle, 2000; Guil-Guerrero et al., 1996; Simopoulos, 2004). It has been also observed that these wild species have healthy substances like vitamins and other antioxidants, minerals, fibre and essential fatty acids, in higher quantities than those found in many cultivated species, so they are a very interesting supplement to basic foods obtained from agriculture and livestock farming. Studies carried out by Rivera et al., (2005) have shown little known key aspects of local foods with plant origin and their potential in different molecular models related to diseases associated to aging (cardiovascular, nervous system, etc.). They also conclude that rural mountain regions in Crete, south of Italy and south of Spain still have healthy habits and store up knowledge about plant resources in process of abandonment that are key elements in the Mediterranean diet, like those that showed significant effects in the study of the seven nations of Keys and collaborators in the sixties of the last century (Keys and Keys, 1959).

The intake of fruits and vegetables or their derived products is now stimulated by the fact that many of their non-nutrient phytochemical compounds are associated to the prevention of diseases (Pandey and Rizvi, 2009; Steinmetz and Potter, 1996). Cell damages caused by the exposure to high levels of free radicals induce cardiovascular diseases, neurological dysfunctions and different types of cancer (Luximon-Ramma et al., 2003). It is already known that fruit and vegetables have a protective action against these diseases because they are a rich source of antioxidants like carotenoids, tocopherols, ascorbates and phenols that scavenge free radicals and reduce the incidence of degenerative pathologies (Prakash et al., 2007; Luximon-Ramma et al., 2003). Moreover, consumers appreciate more and more the replacement of synthetic additives by natural ones, so natural antioxidants found in fruits and vegetables are now a priority area of research (Jayaprakasha et al., 2012). Extracts from herbs or fruits rich in antioxidant compounds are arousing the interest of the alimentary industry because they delay the oxidative degradation of lipids and proteins and, therefore, maintain or increase the nutritional value of foods (Martínez-Tomé et al., 2001; Wojdylo et al., 2007).

The supply of minerals is essential in human diet. Calcium, phosphorous and iron are, in this order, the elements with higher concentrations in the human body. The first

and the second, although they are particularly part of bones and teeth, are also found in intra- and extracellular liquids and they have significant physiological functions. Manganese is needed for the activity of the enzyme superoxide dismutase and selenium for the activity of the enzyme glutathione peroxidase; they are significant elements in the antioxidant mechanism of the organism. During the last decades, low levels of selenium have been related to some types of cancer (Clark et al., 1996). Guil et al. (1996; 1997) and Turan et al. (2003) reported that some wild edible plants had a higher content in calcium, magnesium and potassium than some traditionally employed plants like spinach, pepper and lettuce. Khader and Rama (1998) studied three stages of development of different plants, from which leaves are eaten, in order to determine the best harvesting moment so their intake presents a higher quality in terms of mineral composition.

The term antioxidant can be defined as the substance that in small quantities can avoid or delay the oxidation of easily oxidable materials by acting on the levels of free radicals and reactive species of oxygen that are generated in oxidative reactions (Egea, 2006). The accumulation of these species can cause oxidative damages to DNA, proteins and lipids of cell membranes (lipid peroxidation); these effects are closely related to the processes of aging of tissues and the appearance of certain diseases. The high antioxidant activity of these compounds is mainly due to the fact that they have a strong reducing capacity by oxidising themselves in the presence of oxidant substances and protecting, therefore, the cell structures. Oxidised products from these substances are more stable and less reactive than original oxidant substances. In the organism, there are also some enzymes that later restore them to their reduced state (antioxidant) by using NADH or GSH as source of reducing power (Aruoma, 1996).

There are some synergistic interactions between the different antioxidant nutrients, so their combined action is better than their individual actions. Vitamin E can delay the appearance of the clinic deficiency of selenium and vice versa, because both of them delay the lipid peroxidation. Vitamin C can reduce the oxidised vitamin E, so this one recovers its antioxidant activity. Vitamin E can, at the same time, protect the conjugated bonds of β -carotene, so the activity of β -carotene is higher in the presence of vitamin E. A deficiency of one of them, even a marginal one, can reduce the biological activity of the others (Jacques et al., 1995).

Wild edible plants could have interesting characteristics in their composition that have not yet been described. It has been mentioned that wild plants have a higher concentration of vitamin C than cultivated plants, and studies about the Palaeolithic point out that the quantity of vitamin C obtained by humans by eating wild plants was much higher (about 390 mg/day) than the present average intake in the United States (88 mg/day) (Eaton and Konner, 1985). The researches described by Simopoulos (2004)

conclude that wild plants have higher quantities of vitamins C and E and other antioxidants than cultivated plants.

Zeghichi et al. (2003) described the nutritional composition of 25 wild edible plants from Crete and they observed that all of them had significant quantities of antioxidants and minerals. They found, for example, a high concentration of phenolic compounds in *Crepis vesicaria* L., one of the wild plants consumed in Spain. Schaffer et al. (2005) also found a promising antioxidant activity in *Cichorium intybus*, *Sonchus oleraceus* and *Papaver rhoeas* L., although there were significant differences according to the plant origin and a little clear correlation between the antioxidant activity and the polyphenol content. In Spain, studies of Guil-Guerrero et al. (1997; 1998a, b; 1999; 2003) found, for example, high concentrations of ascorbic acid in *Chenopodium album* L. and *Sonchus oleraceus*, high levels of carotenoids in *Sonchus oleraceus* and *Urtica dioica* L., and a high content of alpha-linolenic acid in the leaves of this last species. Some other works have proposed verdolaga (*Portulaca oleracea* L.) as the plant with the highest content of this essential fatty acid (Simopoulos, 2004). Likewise, Sánchez-Mata et al. (2012) have recently studied the content of vitamin C and organic acids in fifteen wild plants, and they have highlighted the high content of vitamin C of black bryony (near 80 mg/100 g) as well as interesting values in fennel, hop, fiddle dock and bladder campion. Morales et al. (2011) studied in eight of the plants the content of different compounds with antioxidant activity like tocopherols, phenolic compounds and flavonoids, and measured the antioxidant activity using four different techniques. Some of the species showed a high content of the studied compounds and a high antioxidant activity, in particular hop, blink and bladder campion. Morales et al. (2011) found that most of the twenty wild plant species under study had a high percentage of omega-3 fatty acids.

On the other side, a study carried out by Pretel et al. (2004) found that the ascorbic acid content in orange juice correlated significantly with TAA taking into account the 17 analysed varieties and was responsible for 50 to 82 % of the TAA (**Figure 3**). Accordingly, in other citrus fruit juices it has been shown that ascorbic acid accounted for 65-100 % of their antioxidant activity (Cano et al., 1998; Gardner et al., 2000), due to the ascorbic acid ability to efficiently scavenging reactive oxygen species such as O_2 , $OH^\bullet$, peroxy radicals and singlet oxygen, being one of the most important water-soluble antioxidants in cells (Halliwell, 1996). Other bioactive components present in citrus fruits responsible for their antioxidant activity include β -carotene, flavonoids, limonoids, folic acid and dietary fibre (Silalahi, 2002). Accordingly, the pattern of ascorbic acid accumulation was consistent with the increased antioxidant activity in tomato cultivars (De Pascale et al., 2001). However, in other fruit, such as apples, pineapples, strawberries, blueberries, raspberries, blackberries, plums, peaches and nectarines, among others, ascorbic acid made only a small contribution to the TAA, which was

correlated to anthocyanin or phenol content (Gardner et al., 2000; Gil *et al.*, 2002; Kalt et al., 1999; Kim et al., 2003; Wang and Stretch, 2001; Wolfe et al., 2003). Orange fruit has very low concentrations of phenols as compared to other fruits such as cranberries, red grapes, plum, strawberries, cherry, etc. (Vinson et al., 2001) and then, their contribution to TAA might be negligible. In addition, antioxidant activity was correlated with TSS and TA in different ecotypes of small tomatoes and blueberry cultivars, respectively (Scalfi, et al. 2000; Connor et al., 2002). However, any of these correlations were found among the orange varieties of this study. Results show that some traditional orange varieties, such as Guasi, Condesa, Verna, Newhall, Salustiana, Grano de Oro and Capuchina, have an elevated TAA per fruit, even greater than that of Navelina variety, which is actually the most cultivated in Spain. This knowledge could be useful to reconsider the utilisation of certain traditional orange varieties in future breeding programs to help the efficient conservation of an important part of the agricultural biodiversity in the southeast of Spain. In addition, the consumption of orange varieties with high antioxidant activity would improve the beneficial effect on health of these fruit.

Figure 3: Total antioxidant activity (TAA) in orange juice of different orange fruit varieties. Data are the mean $\pm$ SE of determinations made in duplicate in 10 fruits (Adapted from Pretel et al., 2004).

Therefore, different works state that many traditional varieties of fruit trees and wild species have a higher content of antioxidants, vitamins, minerals and omega-3 fatty acids than cultivated plants (Flyman and Afolayan, 2006; Grivetti and Ogle, 2000; Simopoulos, 2004; Pretel et al., 2004). However, there are many wild plants, that have been traditionally consumed and even cultivated, that are unknown in terms of their composition, so their study in comparison to cultivated plants usually consumed would be interesting. This information is valuable not only for ascertaining the nutrient contents that may be used to supplement the nutritional value of the normal diet, but also for environmentalists involved in the design of conservation programmes aimed at protecting and propagating nutritionally valuable wild plant resources.

On the other side, as it has been above mentioned, there is a promotion at international level of the sustainable use of the biological diversity through different programmes that contribute to food security and human nutrition. In this sense, some studies deal with the role of wild edible plants in malnutrition. For example, Nangula et al. (2010) assessed the nutritional value of different wild edible plants and their potential impact on the nutritional state of inhabitants of the Sub-Saharan Africa. These authors concluded that many of these plants are good sources of micronutrients, especially *Manihot esculenta* Crantz, rich in retinol and vitamin C, and *Chenopodium album*, with up to 6 mg/100 g of iron, 18.5 mg/100 g of zinc, 226 mg/100 g of calcium and 211 mg/100 g of magnesium. These plants can help to satisfy the daily needs of these and other essential nutrients, especially in the individuals with a marginal nutritional state. On the other side, some wild plants, like *Arachis hipogaea* L. and *Bidens pilosa* L., are good sources of dietary fibre, while *Urtica dioica* and *Xanthosoma mafaffa* Schott are excellent free radical scavengers. Therefore, the consumption, cultivation and commercialization of these plants must be boosted.

During the last years, there is an increasing interest in nutritional improvement and in the development of healthier foods or foods with specific functional properties (Serrano et al., 2009; Gonzalez-Molina et al., 2012). The studies about the benefits of different compounds of foods (Chen et al., 1992; De Pascale et al., 2004; Łukasz et al., 2012), as well as the increasing interest by the consumers in the effects of food on their health, have led the food industry to offer foods enriched with vitamins, proteins and minerals that respond to the demand by the society of preventing diseases and maintaining a good health (Lavecchia et al., 2013). However, there can be problems regarding the real efficiency of supplements because high doses of a nutrient can cause interactions with a negative effect (Bjelakovi et al., 2012). On the other side, we can now find in the market a great variety of functional products consisting of food matrices to which extracts of botanical species with known functional and nutraceutical properties have been added

(Gonzalez-Molina et al., 2008; Gonzalez-Molina et al., 2009; Gironés-Vilaplana et al., 2012).

A possible application of some wild edible plants with high antioxidant capacity could be their incorporation in fruit matrices in order to increase their value as functional food and/or to increase their stability. Fruits are very important items of a balanced diet, particularly for their role in the prevention of diseases (Peterson et al., 2006; Łukasz et al., 2012), possibly due to their antioxidant potency and their effect on plasma lipid metabolism (Jung et al., 2006). The benefits for health of some fruits have been mainly attributed to the presence of bioactive compounds like phenolic compounds (Goulas and Manganaris, 2012), ascorbic acid (Klimczak et al., 2007) and carotenoids (Wang et al., 2008). Some prior studies have shown that these beneficial properties can be increased by adding natural antioxidant sources, like the enrichment of lemon juice with *Aronia melanocarpa* (Gonzalez-Molina et al., 2008).

On the other side, edible plant oils are obtained from the oil found in seeds and fruits that later undergoes a process of physical and chemical refining that drastically changes the content of phenolic compounds (Owen et al., 2000). Moreover, oils and fats suffer significant oxidative changes when they are subject to high temperatures during storage or cooking that reduce their nutritional quality. Antioxidants can increase the shelf-life of oils by protecting them against the degradation caused by oxidation that produces rancidity and changes in colour. One of the biological activities of phenols found in edible oils is their antioxidant activity, which is related to their shelf-life (Servili et al., 2004). Some researchers (Marinova et al., 2001) found that some phenols with high antioxidant capacity are little stable at high temperatures. More recently, Lee *et al.* (2006) stated the need to add antioxidants in order to improve the oxidative stability of edible oils. Synthetic antioxidants are commonly employed, like the butylhydroxytoluene (BHT); however, they have toxic and carcinogenic effects on human beings so their utility is limited.

Considering this situation, there is a need to find effective antioxidants with natural origin that can be employed in foods for avoiding lipid oxidation. It has been also shown that the antioxidant properties of some plants and spices delay the rancidity in oils and fatty foods (Bracco et al., 1981; Bera et al., 2006). It is known that natural extracts from some herbs, spices and vegetables are stable against autoxidation due to the presence of natural phenolic compounds (Boskou, 2006; Ammari et al., 2012). Lindberg and Bertelsen (1995) mentioned the antioxidant effect of chemical compounds found in oregano (*Origanum vulgare* L.). Abdalla and Roozen (1999) studied the antioxidant activity of different plant extracts on sunflower oil under conditions of accelerated oxidation (60 °C), and they found that these extracts have a higher antioxidant activity than BHT although, depending on the extraction method, they could act as prooxidants. Ullah et al.

(2003) showed that the natural antioxidants found in red pepper had an inhibitory effect on the oxidation induced by light in soya and sunflower oils, although BHT was generally more effective than natural antioxidants (Ullah et al., 2003).

4. ENZYMATIC PEELING OF CITRUS FRUITS

The conventional industrial processes for the peeling of citrus fruits include the manual or mechanical separation of the rind and the later chemical degradation of the rests of albedo and segment membranes. This method implies a high cost of labour, high volumes of water for washing and causes significant environmental problems due to the use of peeling caustics. The enzymatic peeling is an alternative method for removing the rind of the fruits with lower water consumption and causing less contamination than traditional processes. The principle of the enzymatic peeling is based on the digestion, by an enzymatic preparation, of pectic substances found in cell walls of plants (Bruemmer et al., 1978; Berry et al., 1988). The process of enzymatic peeling of citrus fruits, clearly explained in **Figure 4**, starts with a selection of fruits followed, in some cases, by a washing with chlorinated water and, in certain cases, by a later treatment with hot water or scalding. Then, some incisions are made on the flavedo (coloured area of the skin) to allow the entry of the enzymatic solution in the albedo (white and soft area around segments) when vacuum is applied. Fruits are later put into a container with an enzymatic solution and vacuum is applied. Fruits are kept in incubation with the enzyme, washed with pressurised water and the finished product is obtained (whole fruit or segments).

From the molecular point of view, pectin, cellulose and hemicellulose are responsible for the adherence of the skin to the fruit (Whitaker, 1984). Therefore, both pectinases and cellulases are needed for the enzymatic peeling. The cellulases are probably needed for the release of the pectins in the albedo, and the pectinases contribute to the hydrolysis of the polysaccharides of the cell wall (Ben-Shalom et al., 1986; Coll, 1996). Bruemmer et al., in 1978, were the first authors employing this enzymatic method in the peeling process of grapefruit by vacuum infusion of commercial pectolytic preparations, showing that the obtained sections maintained their original taste and texture with higher efficiency and quality than those obtained by conventional peeling processes. They also observed how the commercial pectinases considerably differ on their peeling efficiency. Likewise, Berry et al. (1988) proved, using the method developed by Bruemmer et al. (1978); that in segments and entire grapefruits enzymatically peeled, the loss of juice was lower than in fruits obtained by conventional chemical or manual methods. In order to obtain a better knowledge of the processes of enzymatic degradation, Ben Shalom et al. (1986) stated the importance of evaluating the effect of commercial enzymes on the substrates needed to be degraded. This fact was later

confirmed by other authors (Rouhana and Mannheim, 1994; Soffer and Mannheim, 1994; Baker and Wicker, 1996; Pretel et al., 2005; Pinnavaia et al., 2006). However, not only the enzymatic preparation is critical for obtaining a good peeling efficiency, there are many other determining parameters. For instance, the conditions of adherence of the peel to the fruit and its thickness are different according to the species or the citrus varieties; and the design of the cuts, the conditions of vacuum, temperature and pH will influence the separation between the albedo and the peel of the segments (Berry et al., 1988; McArdle and Culver, 1994; Pretel et al., 1997; Pretel et al., 1998 a;b; Prakash et al., 2001; Suutarinen et al., 2003; Liu et al., 2004; Pagán et al., 2005; Pretel et al., 2007a;b). On the other side, the reuse of the enzymatic solution has been studied by different authors since, from the economical point of view; this is one of the determining factors when using the process at an industrial level (Pretel et al., 1997; Rouhana and Mannheim, 1994; Pagán et al., 2005; Pretel et al., 2007b).

Figure 4: Process of enzymatic peeling of citrus fruits (Pretel et al., 2009).

All citrus fruits show the same anatomical structure (**Figure 5a**), although the elements of this structure are different depending on the species and variety. The epicarp consists of the coloured portion of the peel and it is known as the flavedo. In the flavedo there are cells containing the carotenoids, which give the characteristic colour to the different citrus fruits, i.e., orange, mandarin or tangerine, grapefruit and lemon, among others. The oil glands, also found in the flavedo, are the raised structures in the skin or the citrus fruit that contain the essential oils characteristic of each citrus cultivar. Immediately under the epicarp is the mesocarp or albedo. This is typically a thick, white, spongy layer. However, in some varieties of orange and mandarins the albedo is very thin and difficult to separate from the flavedo, a fact affecting the enzymatic peeling. The albedo consists of large parenchymatous cells rich in pectic substances and hemicelluloses (Ting and Rouseff, 1986). It completely envelopes the endocarp, the usual edible portion of citrus fruits.

The combined albedo and flavedo are called the pericarp, commonly known as the rind or peel. The endocarp is made up of a set of vesicles containing juice and grouped in the segments that are found in a number between 5 and 19, depending on the species. Oranges usually have between 9 and 11 segments, while lemons have between 8 and 11, and grapefruits between 12 and 15. The number of seeds varies depending on species, variety and pollination conditions. However, most cultivated varieties do not have seeds and these are the most suitable for the enzymatic peeling since they are the most appreciated by the consumers (Pretel et al., 2008; 2009). In some orange varieties, a secondary small and atrophied orange that resembles a navel is developed in the base of the fruit (**Figure 5b**).

The “navel” and some morphological characteristics like the adhesion between albedo and segments, the adhesion between segments and, above all, the homogeneity of the segment membrane are characteristic of each species and variety (Pretel et al., 2007a), and they directly influence the process of enzymatic peeling. The presence of the navel hampers the diffusion of the enzymatic solution in the tissue and the irregularity of the segments decreases the quality of the final product, as it could be verified in the variety Thomson (Pretel et al., 2007a). The high adhesion between the albedo and the segments and the close union between these last ones increase the levels of vacuum needed for the process because the diffusion of the enzymatic solution in the tissues would be hampered (Pretel et al., 1997). The presence of fractures in the carpelar membrane and, therefore, the lack of homogeneity of the skin, make it difficult to obtain segments since the enzymatic solution penetrates even with low levels of vacuum (Pretel et al., 1997).

Figure 5: Transverse cut of a citrus fruit (a). Longitudinal cut of an orange Navel (b). (Pretel et al., 2009).

Most studies about enzymatic peeling of citrus fruits have been carried out using different varieties of oranges and grapefruits, although other species like mandarin, lemon and cimboba have been also studied. The orange (*Citrus sinensis*) is the most significant species of the genus *Citrus* because it is the most consumed one. The fruits differ in form and colour according to the varieties (Pretel et al., 2004; Lousert, 1992).

It is interesting, from an economic point of view, to know the thickness of the albedo since the quantity of enzymatic solution needed for the process will be higher if the fruit has a higher quantity of albedo (McArdle and Culver, 1994). On the other side, a too much thin albedo could not absorb enzymatic solution enough for an adequate fruit peeling. Although the thickness of the albedo can give an idea about the quantity of enzymatic solution each species or variety can absorb, its degree of compaction is one of the parameters affecting the enzymatic peeling (Pretel et al., 1997) because it directly influences the ease of penetration of the pectolytic enzymes solution when vacuum is applied. The enzymatic solution better diffuses when the tissue is porous, that is, when the parenchymatous tissue of the albedo shows wide intercellular spaces and, therefore, the volume of the albedo is higher than its weight (Baker and Bruemmer, 1989). Then, when vacuum is applied, the air in the intercellular spaces is more easily replaced by the enzymatic solution. The species and

varieties with a less porous albedo needs harder vacuum conditions for the enzymatic solution to penetrate in the albedo because the compaction of the tissue hampers the distribution of the enzymatic solution among the cells (Pretel et al., 1997). **Figure 6** shows the thickness of albedo in different traditional varieties of orange. Most varieties show an albedo thickness between 0.10 and 0.15 mm. Only the varieties Común, Salustiana and the three from the group Navel exceed this value. Therefore, more enzymatic solution would be needed for the enzymatic peeling of these five varieties.

Figure 6: Thickness of the albedo in different traditional varieties of orange. Data are the mean $\pm$ SE of the determinations in 10 fruits (Adapted of Valdés et al., 2005).

On the other side, not all citrus fruits are adequate for obtaining segments using enzymatic peeling because due to some morphological characteristics, like albedo compaction or fragility of the peel of the segments, could hamper this process. **Figure 7** shows some morphological characteristics of the 17 varieties of orange that can affect their suitability for enzymatic peeling. The strong adhesion between albedo and segments hampers the process of enzymatic peeling (Pretel et al., 1997; Valdés et al., 2005). In this sense, the varieties of orange with more difficulties are Capuchina, Newhall and Sanguinelli because the albedo and the segments are strongly adhered. Therefore, the close union between these segments should increase the levels of vacuum needed for the process because the diffusion of the enzymatic solution in the tissues would be hampered (Pretel et

al., 1997). The varieties with the strongest union of the segments and that, therefore, should present more resistance to penetration of the enzymatic solution, would be the three varieties of the group navel and the variety Cadenera. The rest of varieties show acceptable values, equal or higher than 3. On the other side, the homogeneity of the surface of the skin of the segments is significant for peeling as the fractures in the skin would cause the penetration of the enzymatic solution in the segments when vacuum is applied (Pretel et al., 1997). The varieties with the less homogenous surface of the segment skin are Sanguinelli, Capuchina and Newhall, so the obtaining of quality segments is quite difficult. Therefore, and considering the three morphological parameters analysed, the best varieties for obtaining segments using enzymatic peeling are Común, Común de Orihuela, Salustiana and Cadenera.

Figure 7: Characteristics of fruits influencing enzymatic peeling: union albedo-segments (●), union segment-segment (▼) and homogeneity of the surface of the segment skin (■) of different orange varieties. Data are the mean $\pm$ SE of the determinations in 10 fruits (Adapted of Valdés et al., 2005).

5. REFERENCES

- Abdalla, A.E. and Roozen, J.P. (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chemistry*. 64 (3): 323-329.
- Ammari, F., Cordella, C. B. Y., Boughanmi, N. and Rutledge, D.N. (2012). The increase in oxidative stability of sunflower oil enriched with *Nigella sativa* L. seed extracts. *Food Measure*. 6: 12 20.
- Arsuaga, J.L. and Martínez, I. (1998). La especie elegida. Ediciones Temas de Hoy. Madrid.
- Aruoma, O.I. (1996). Eat, drink and be healthy. *Chem. Br*. 32: 29-31.
- Bach-Faig, A., Berry, E.M., Lairon, D., Reguant, J., Trichopoulou, A., Dernini, S., Medina, F.X., Battino, M., Belahsen, R., Miranda, G. and Serra-Majem, L. (2011). Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutr*. 14:2274-84.
- Baker, R.A. and Bruemmer, J.H. (1989). Quality and stability of enzymatically peeled and sectioned citrus fruit. In: *Quality Factors of Fruits and Vegetables*, American Chemical Society, ed. J.J. Jen, Washington, DC, pp. 140-148.
- Baker, R.A. and Wicker, L. (1996). Current and potencial applications of enzyme infusion in the food industry. *Trends in Food Science and Technology*. 7: 279-284.
- Barret, H.C. and Rhodes, A.M. (1976). A numerical Taxonomic Study of Affinity Relationships in Cultivated Citrus and Its Close Relatives. *Systematic Botany*, 1(2): 105-136.
- Benítez, G. (2009). Etnobotánica y Etnobiología del poniente granadino. Tesis doctoral. Facultad de Farmacia. Universidad de Granada.
- Ben-Shalom, M., Levi, A. and Pinto, R. (1986). Pectolytic enzyme studies for peeling of grapefruit segment membrane. *Journal of Food Science*. 51 (2): 421-423.
- Bera, D., Lahiri, D. and Nag, A. (2006). Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants. *Journal of Food Engineering*. 74 (4): 542 545.
- Berry, R.E., Baker, R.A. and Bruemmer, J.H. (1988). Enzyme separated sections: A new lightly processed citrus product. In: *Proceedings of the Sixth International Citrus Congress*, ed. R. Goren and K. Hendel, Tel-Aviv, Israel, Balaban Publishers, Philadelphia, pp. 1711-1716.
- Bharucha, Z. and Pretty, J. (2010). The roles and values of wild foods in agricultural systems. *Phil Trans R Soc B*. 365(1554): 2913-2926.
- Bjelakovic G., Nikolova, D., Glud, L.L., Simonetti, R.G. and Glud, C. (2012). Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases (Review). The Cochrane Collaboration. Published by JohnWiley and Sons, Ltd. Issue 3. <http://www.thecochranelibrary.com>.
- Boskou, D. (2006). Sources of natural phenolic antioxidants. *Trends in Food Science and Technology*. 17: 505 512.
- Bracco, U., Loliger J. and Viret J. (1981). Production and use of natural antioxidant. *J Am OilChem Soc*. 58: 686-690.

- Bruemmer, J.H., Griffin, A.W. and Onayami, O. (1978). Sectionizing grapefruit by enzyme digestion. *Proc. Florida State Hort. Soc.* 91:112-114.
- Calabrese, F. (1973). Considerazioni sulla classificazione botanica delle Aurantioideae (*Rutaceae*). *Webbia*. 28(1): 161-187.
- Cano, A., Hernández-Ruíz, J., García-Cánovas, F., Acosta, M. and Arnao, M.B. (1998). An end-point method for estimation of the total antioxidant activity in plant material. *Phytochemical Analysis*. 9: 196-202.
- Carbonell, E. and Pastó, I. (2005). La alimentación de nuestros ancestros. En: Salas-Salvadó J, García-Lorda P, Sánchez Ripollés JM (ed.) *La alimentación y la nutrición a través de la historia*. Glosa, Barcelona, pp 17-34.
- Chen J, Geissler, C. and Parpia, B. (1992). Antioxidant status and cancer mortality in China. *Int J Epidemiol*. 21:625 - 635.
- Clark, L.C., Combs, G.F., Turnbull, B.W., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Glover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Leshner, J.L. Park, H.K., Sanders, B.B., Smith, C.L. and Taylor, J.R. (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA*. 276(24):1957-63.
- Coll, L. (1996). Polisacáridos estructurales y degradación enzimática de la membrana carpelar de mandarina Satsuma (*Citrus unshiu* Marc.). Pelado enzimático de los segmentos. Universidad de Murcia, Murcia.
- Connor, A.M., Luby, J.J., Hancock, J.F., Berkheimer, S. and Hanson, E.J. (2002). Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *Journal of Agricultural and Food Chemistry*. 50: 893-898.
- Cordain, L., Boyd, S., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B.A., O'Keefe, J.H. and Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st. century. *Am J Clin Nutr*. 81(2): 341-354.
- De Pascale, S., Maggio, A., Fogliano, V., Ambrosino, P. and Ritieni, A. (2001). Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *Journal of Horticultural Science and Biotechnology*. 76: 447-453.
- Eaton, S.B. and Konner, M. (1985). Paleolithic Nutrition - A Consideration of Its Nature and Current Implications. *New England Journal of Medicine*. 312(5): 283-289.
- Egea, J.M. and Egea, J.M. (2010). Agricultura ecológica y biodiversidad agraria. *Ae* 0: 12-16.
- Egea, I., Sánchez-Bel, P., Romojaro, F. and Pretel, M.T. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods For Human Nutrition*. 65: 121-129.
- Egea, I. (2006). Influencia de diferentes métodos de conservación sobre la calidad y sistemas antioxidantes de alimentos vegetales. Tesis Doctoral. Universidad de Murcia.
- Ertug, F. (2004). Wild edible plants of the Bodrum Area (Mugla Turkey). *Turkish Journal of Botany*. 28: 161 174.
- Esquinas-Alcázar, J.T. (2007). Proteger la diversidad genética de los cultivos para la seguridad alimentaria: desafíos políticos, éticos y técnicos. En: *Biodiversidad y derecho a la alimentación*. Urgente, pp. 9-19.

- Esquinas-Alcázar, J.T. (1993). La diversidad genética como material básico para el desarrollo agrícola. En: La Agricultura del Siglo XXI. Cubero JI y Moreno MT (coord.). Mundi-Prensa. Madrid.
- Esquinas-Alcazar, J.T. (1981). Recursos fitogeneticos de la region Andina. (Consejo Internacional de Recursos Fitogeneticos (CIRF)) In: Plant Genetic Resources Newsletter (IBPGR/FAO).47: 36-39.
- Fajardo, J., Verde, A., Rivera, D., Obón, C. (2007). Etnobotánica en la Serranía de Cuenca. Las plantas y el hombre. Diputación de Cuenca.
- FAO (Food and Agriculture Organization of the United Nations). (2011). Second Global Plant of Action for Plant Genetic Resources for Food and Agriculture Adopted by the FAO Rome, Italy.
- FAO (Food and Agriculture Organization of the United Nations). (1996). Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture. FAO, Rome, Italy.
- Flyman, M.V. and Afolayan, A.J. (2006). The sustainability of wild vegetables for alleviating human dietary deficiencies. S Afr J Bot. 72: 492-497.
- Frassetto, L.A., Schloetter, M., Mietus-Synder, M., Morris, R.C. and Sebastian, A. (2009). Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. European Journal of Clinical Nutrition. 63(8): 947-955.
- Gardner, P.T., White T.A.C., McPhail D.B. and Duthie, G.G. (2000). The Relative Contributions of Vitamin C, Carotenoids and Phenolics to the Antioxidant Potential of Fruit Juices. Food Chemistry. 68: 471-474.
- Gil, M.I, Tomás-Barberán, F.A., Hess-Pierce, B. and Kader, A.A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. Journal of Agricultural and Food Chemistry. 50: 4976-4982.
- Gironés-Vilaplana, A., Mena, P., Garcíaa-Viguera C. and Moreno, D.A. (2012). A novel beverage rich in antioxidant phenolics: Maqui berry (*Aristotelia chilensis*) and lemon juice. LWT- Food Sci Technol. 47:279-286.
- González-Molina, E., Gironés-Vilaplana, A., Mena, P. Moreno, D. and García-Viguera, C. (2012). New Beverages of Lemon Juice with Elderberry and Grape Concentrates as a Source of Bioactive Compounds. 77 (6): 727 C733.
- González-Molina E., Moreno D.A. and García-Viguera C. (2009). A new drink rich in healthy bioactives combining lemon and pomegranate juices. Food Chem. 115:1364 72.
- González-Molina E., Moreno, D.A. and García-Viguera C. (2008). Aronia-enriched lemon juice: a new highly antioxidant beverage. J Agric Food Chem. 56:11327 33.
- Goulas, V. and Manganaris, G.A.. (2012). Exploring the phytochemical content and the antioxidant potential of Citrus fruits grown in Cyprus Food Chemistry. 131:39 47.
- Grivetti, L.E. (2006). Edible wild plants as food and medicine: reflections on thirty years of fieldwork. En: Pieroni A, Price LL (ed.) Eating and Healing. Traditional food as medicine. Food Product Press, Haworth Press, New York, pp 11-38.
- Grivetti, L.E. and Ogle, B.M. (2000). Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutr Res Rev. 13: 31-46.

- Guil-Guerrero, J.L., Reboloso-Fuentes, M.M. and Torija-Isasa, M.E. (2003). Fatty acids and carotenoids from Stinging Nettle "*Urtica dioica* L.). J Food Compos Anal. 16(2): 111-119.
- Guil-Guerrero, J.L., Giménez-Martínez, J.J. and Torija-Isasa, M.E. (1999). Nutritional composition of wild edible crucifer species. J Food Biochem. 23(3): 283-294.
- Guil-Guerrero, J.L., Gimenez-Gimenez, A., Rodríguez-García, I. and Torija-Isasa, M.E. (1998a). Nutritional composition of *Sonchus* species (*S. asper* L., *S. oleraceus* L. and *S. tenerrimus* L.). J Sci Food Agric. 76(4): 628-632.
- Guil-Guerrero, J.L., Giménez-Martínez, J.J. and Torija-Isasa, M.E. (1998b). Mineral nutrient composition of edible wild plants. J Food Compos Anal. 11: 322-328.
- Guil-Guerrero, J.L., Rodríguez-García, I. and Torija-Isasa, M.E. (1997). Nutritional and toxic factors in selected wild edible plants. Plant Foods Hum Nutr. 51(2): 99-107.
- Guil-Guerrero, J.L., Torija-Isasa, M.E., Giménez-Martínez, J.J. and Rodríguez-García, I. (1996). Identification of fatty acids in edible wild plants by gas chromatography. J Chromatogr. A. 719: 229-235.
- Halliwel, B. (1996). Antioxidants in human health and disease. Annual Review of Nutrition. 16: 33-50.
- Heinrich, M., Walter E. Muller, W.E. and Claudio Galli, C. (2010). Local Mediterranean Food Plants And New Nutraceuticals. S. Karger AG. Pp 285.
- Heinrich, M., Leonti, M., Nebel, S. and Peschel, W. (2005). Local food-nutraceuticals: an example of a multidisciplinary research project on local knowledge. J Physiol Pharmacol. 56 Suppl. 1:5-22.
- Heywood, V. (2011). Ethnopharmacology, food production, nutrition and biodiversity conservation: Towards a sustainable future for indigenous peoples. Journal of Ethnopharmacology. 137: 1 15.
- Heywood, V. (2009). Introduction: perspectives for economically important wild species and neglected crops in the Mediterranean. Boccone. 23: 107 114.
- Izco, S.J. et al. (2004). Botánica. 2ª edición Ed. McGrawHill-Interamericana de España. Madrid.
- Jacques, P.F, Halpner A. D. and Blumberg J.B. (1995). Influence of combined antioxidant nutrient intakes on their plasma concentrations in an elderly population. Am. J. Clin. Nutr. 62: 1228-1233.
- Jayaprakasha, G.K., Murthy, K.N.C., Etlinger, M., Mantur, S.M., and Patil, B.S. (2012). Radical scavenging capacities and inhibition of human prostate (LNCaP) cell proliferation by *Fortunella margarita*. Food Chemistry. 131:184-191.
- Jonsson, T., Granfeldt, Y., Ahren, B., Branell, U.C., Palsson, G., Hansson, A., Soderstrom, M. and Lindeberg, S. (2009). Beneficial effects of a Paleolithic diet on cardiovascular risk factors in type 2 diabetes: a randomized cross-over pilot study. Cardiovascular Diabetology. 8:35.
- Jonsson, T., Ahrén, B., Pacini, G., Sundler, F., Wierup, N., Steen, S., Sjöberg, T., Ugander, M., Frostegård, J., Goransson, L. and Lindeberg, S. (2006). A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs. Nutrition and Metabolism. 3:39.

- Jung, U. J., Lee, M. K., Park, Y. B., Kang, M. A. and Choi, M. S. (2006). Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *The International Journal of Biochemistry and Cell Biology*. 38: 1134-1145.
- Kalt, W., Forney, C.F., Martin, A. and Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry*. 47: 4638-4644.
- Keys, A. and Keys, M. (1959). *Eat well and stay well*. New York. Doubleday and Company.
- Khader, V. and Rama, S. (1998). Selected mineral content of common leafy vegetables consumed in India at different stages of maturity, *Plant foods for Human Nutrition*. 53: 71-78.
- Kim, D.-O., Jeong, S.W. and Lee, C.Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*. 81: 321-326.
- Kimball, D.A. (1999). *Citrus processing. A complete guide*. Gaithersburg, MD: Aspen Publishers, Inc.
- Klimczak, I., Malecka, M., Szlachta, M. and Gliszczynska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis*. 20:313-322.
- Konner, M. and Eaton, S.B. (2010). Paleolithic Nutrition Twenty-Five Years Later. *Nutrition in Clinical Practice*. 25(6): 594-602.
- Kuhnlein, H.V. (2009). Introduction. Why are indigenous peoples' food systems important and why do they need documentation? In: Kuhnlein, H.V., Erasmus, B., Spigelski, D. (Eds.), *Indigenous Peoples' Food Systems: The Many Dimensions of Culture, Diversity and Environment for Nutrition and Health*. United Nations Food and Agriculture Organization, Rome, pp. 1-4.
- Lavecchia, T., Rea, G., Antonacci, A. and Giardi, M.T. (2013). Healthy and adverse effects of plant-derived functional metabolites: The need of revealing their content and bioactivity in a complex food matrix. *Critical Reviews in Food Science and Nutrition*. 53: 198-213.
- Lee, M.R., Lin, C.Y., Li, Z.G. and Tsai, T.F. (2006). Simultaneous analysis of antioxidants and preservatives in cosmetics by supercritical fluid extraction combined with liquid chromatography mass spectrometry. *Journal of Chromatography A*. 1120 (1-2): 244-251.
- Lindberg, M. and Bertelsen, G. (1995). Spices as antioxidant. *Trends in Food Science and Technology*. 6: 271-277.
- Liu, F., Osman, A., Yuso, F.S. and Ghazali, H.M. (2004). Effects of enzyme-aided peeling on the quality of local mandarin (*Citrus reticulata* B.) segments. *Journal of Food Processing and Preservation*. 28: 336-347.
- Loussert, R. (1992). *Los agrios*. Mundiprensa, Madrid.
- Luximon-Ramma, A., Baborun, T. and Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritanian exotic fruits. *J Sci Food Agric*. 83:496-502.
- Łukasz, L., Pieroni, A., Tardío, J., Pardo-de-Santayana, M., Söukand, R., Svanberg, I. and Kalle, R. (2012). Wild food plant use in 21st century Europe: the disappearance of

- old traditions and the search for new cuisines involving wild edibles. *Acta Soc Bot Pol.* 81(4):359-370.
- Marinova, E., Yanishlieva N., Toneva A., Psomiadou E. and Tsimidou M. (2001). Los cambios en la estabilidad a la oxidación y el contenido de tocoferol en los aceites durante el calentamiento por microondas. *Riv. Ital. Sostanze Grasse.* 78: 529-533.
- Martinez-Tome, M., Jimenez, A., Ruggieri, S., Frega, N., Strabbioli, R. and Murcia, M. (2001). Antioxidant properties of mediterranean spices compared with common food additives. *Journal of Food Protection.* 64: 1412-1419.
- Mazhar, F., Buckles, D., Satheesh, P.V. and Akhter, F. (2007). Food Sovereignty and Uncultivated Biodiversity in South Asia: Essays on the Poverty of Food Policy and the Wealth of the Social Landscape. Academic Foundation, New Delhi in association with IDRC, Ottawa.
- McArdle, R.N. and Culver, C.A. (1994). Enzyme infusion: A developing technology. *Food Technology.* 11: 85-89.
- Mesa, S. (1996). Estudio Etnobotánico y Agroecológico de la comarca de la Sierra de Mágina (Jaén). Tesis doctoral. Universidad Complutense de Madrid.
- Misra, S., Maikhuri, R.K., Kala, C.P., Rao, K.S. and Saxena, K.G. (2008). Wild leafy vegetables: a study of their subsistence dietetic support to the inhabitants of Nanda Devi Biosphere Reserve, India. *Journal of Ethnobiology and Ethnomedicine.* 4: 4-15.
- Morales, R., Tardío, J., Aceituno, L., Molina, M. and Pardo-de-Santayana, M. (2011). Biodiversidad y Etnobotánica en España. En: Viejo-Montesinos J.L. (ed.) "Biodiversidad. Aproximación a la diversidad botánica y zoológica de España". Real Sociedad Española de Historia Natural, Madrid, pp 157-207.
- Mulet, L. (1991). Estudio etnobotánico de la provincia de Castellón. Servicio de Publicaciones de la Diputación de Castellón. Castellón.
- Nangula, P., Uusiku, A., Oelofse, K., Duodu, G., Megan, J. and Bester, M. (2010). Mieke faber nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis.* 23: 499-509.
- Ortiz, J.M. (1985). Nomenclatura botánica de los cítricos. *Levante Agrícola.* 250-60: 71-79.
- Owen R.W., Mier W. and Giacosa A. (2000). Identification of lignans as major components in the phenolic fraction of olive oil. *Clim Chem.* 46: 976-988.
- Pagán, A., Ibarz, A. and Pagán, J. (2005). Kinetics of the digestion products and effect of temperature on the enzymatic peeling process of oranges. *Journal of Food Engineering.* 71: 361-365.
- Padulosi, S., Hodgkin, T., Williams, J.T. and Haq, N. (2002). Underutilised crops: trends, challenges and opportunities in the 21st Century. In, Engels, J., Rao, V.R. and Jackson, M. (eds.) *Managing plant genetic diversity.* Wallingford, UK, CAB International, pp 323-338.
- Pandey, K.B. and Rizvi, I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev.* Nov-Dec; 2(5): 270-278.
- Pardo de Santayana, M., Tardío, J. and Morales, R. (2005). The gathering and consumption of wild edible plants in the Campoo (Cantabria, Spain). *Int J Food Sci Nutr.* 56(7): 529-542.

- Pelagic, V. (1893). Folks teacher IV. (in Serbian), Matica Srpska, Novi Sad.
- Pereira, J. and García, G. (2002). Bellota, el alimento de la edad de oro, *Arqueoweb* 4 (2).
- Peterson, J. J., Beecher, G. R., Bhagwat, S. A., Dwyer, J. T., Gebhardt, S. E. and Haytowitz, D.B.(2006). Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from the analytical literature. *Journal of Food Composition and Analysis*. 19: 574-580.
- Pina (1989). Los cítricos en ornamentación. *Levante agrícola*. 293-4 (3): 179-183.
- Pinnavaia, S., Baldwin, E.A., Plotto, A., Narciso, J. and Senesi, E. (2006). Enzyme-peeling of Valencia oranges for fresh-cut slices. *Proceedings of the Florida State Horticultural Society*. 119: 335-339.
- Pistorius, R. (1997). Scientists, plants and politics - A history of the plant genetic resources movement, International Plant Genetic Resources Institute, Roma. Pp 134.
- Pochettino, M.L. (2005). Verduras en Europa, Yuyos en América: Prácticas y Conocimientos sobre Malezas comestibles. Asociación de Amigos del Archivo General de la Provincia Esperanza. Santa Fe. República Argentina.
- Prakash, D., Upadhyay, G., Gupta, C., Pushpangadan, P. and Singh, K.K. (2012). Antioxidant and free radical scavenging activities of some promising wild edible fruits *International Food Research Journal*. 19 (3): 1109-1116.
- Prakash, D., Suri, S., Upadhyay, G. and Singh, B.N. (2007). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. *International Journal of Food Sciences and Nutrition*. 58: 18-28.
- Prakash, S., Singhal, R.S. and Kulkarni, P.R. (2001). Enzymic peeling of Indian grapefruit (*Citrus paradisi*). *Journal of the Science of Food and Agriculture*. 81: 1440-1442.
- Pretel, M.T., Murcia, R., Romojaro, A., and Obón, C. (2014). Estudio Etnobotánico de Plantas Silvestres Comestibles de Sierra Espuña (Murcia). II Congreso Estatal de Agricultura Ecológica Urbana y Periurbana. Marzo. *In press*.
- Pretel, M.T., Sánchez-Bel, P., Egea, I. and Romojaro, F. (2009). Enzymatic peeling of citrus fruits. (Review Invited). En: *Enzymes in Fruit and Vegetable Processing. Enzymes in fruit and Vegetable Processing: Chemistry and Engineering Applications*. CRC Press/Taylor and Francis. Chapter Six, 145-172.
- Pretel, M.T., Sanchez-Bel, P., Egea, I. and Romojaro, F. (2008). Enzymatic peeling of citrus fruits: factors affecting degradation of the albedo. In: *Tree and forestry Science and Biotechnology*, ed. Teixeira da Silva J.A. Global Science Books, Islework, UK, 52-59.
- Pretel, M.T., Botella, M.A., Amorós, A., Zapata, P.J. and Serrano, M. (2007a). Optimization of vacuum Infusion and incubation time for enzymatic peeling of 'Thomson' and 'Mollar' oranges. *LWT-Food Science and Technology*. 40: 12-30.
- Pretel, M.T., Botella, M.A., Amorós, A., Serrano, M., Egea, I. and Romojaro, F. (2007b). Obtaining fruit segments from a traditional orange variety (*Citrus sinensis* (L.). Osbeck cv. Sangrina) by enzymatic peeling. *European Food Research and Technology* 225: 783-788.
- Pretel, M.T., Amorós, A., Botella, M.A., Serrano, M. and Romojaro, F. (2005). Study of albedo and carpelar membrane degradation for further application in enzymatic peeling of citrus fruits. *Journal of the Science of Food and agriculture*. 84: 86-90.

- Pretel, M.T., Botella, M.A., Zapata, P.J., Amorós, A. and Serrano, M. (2004). Antioxidant activity and general fruit characteristics in different traditional orange (*Citrus sinensis* (L.) Osbeck) varieties. *European Food Research and Technoogy*. 219: 474-478.
- Pretel, M.T., Romojaro, F., Serrano, M., Amorós, A., Botella, M.A. and Obón, C. (2001). Nuevos usos comerciales para variedades tradicionales de cítricos del Sureste español. *Levante agrícola*. 357: 320-325.
- Pretel, M.T., Fernández, P.S., Martínez, A. and Romojaro, F. (1998a). The effect of modified atmosphere packaging on “ready-to-eat” oranges. *Lebensmittel Wissencharnd Und Technology*. 31: 322-328.
- Pretel, M.T., Fernández, P.S., Martínez, A. and Romojaro, F. (1998b). Modelling design of cuts for enzymatic peeling of mandarin and optimization of different parameters of the process. *Z Lebensmittel Unterssuehung Und Firshung*. 207: 322-327.
- Pretel, M.T., Lozano, P., Riquelme, F. and Romojaro, F. (1997). Pectic enzymes in fresh fruit processing: optimization of enzymic peeling of oranges. *Process Biochemistry*. 32 (1): 43-49.
- Ranfa, A., Maurizi, A., Romano, B. and Bodesmo, M. (2013). The importance of traditional uses and nutraceutical aspects of some edible wild plants in human nutrition: the case of Umbria (central Italy), *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana*, DOI: 10.1080/11263504.2013.770805. Published online: 25 Feb 2013.
- Rivera, D. and Obón, C. (2007). *Manual de Teoría y Prácticas. Etnobotánica*. <http://ocw.um.es/ciencias/etnobotanica/Material%20de%20clase/etnobotanica-capitulo15>. Accessed April 2014.
- Rivera, D. Verde, A. Fajardo, J. Inocencio, C. Obón, C. and Heinrich, M. (2006a). Guía etnobotánica de los alimentos locales en la provincia Albacete. Ed. Instituto de estudios Albacetenses “Don Juan Manuel” de la EXCMA. Diputación de Albacete.
- Rivera D., Obón C., Heinrich M., Inocencio C., Verde A. and Fajardo, J. (2006b). Gathered Mediterranean Food Plants- Ethnobotanical investigations and historical development. In Heinrich M, Müller WE and Galli C, eds. *Local Mediterranean Food Plants and Nutraceuticals*. Basel, Karger: Forum Nutrition, 18-74.
- Rivera, D., Obón, C., Inocencio, M., Heinrich, M., Verde, J., Fajardo, J. and Llorach, R. (2005). The Etnobotanical Study of Local Mediterranean Food Plants as Medicinal Resources in Southern Spain. *Journal of Physiology and Pharmacology*. 56 (Suppl 1): 97-114.
- Rivera, D., Obón, C., Ríos, S., Selma, C., Méndez, F., Verde, A., and Cano, F. (1998). Las variedades tradicionales de frutales de la cuenca del río Segura. Catálogo etnobotánico. Cítricos, frutos carnosos y vides. Ed. Diego Marín. Murcia. Pp. 263.
- Rouhana, A and Mannheim, C.H. (1994). Optimization of enzymatic peeling of grapefruit. *Lebensmittel Wissencharnd Und Technology*. 27: 103-107.
- Ruiz-Rodríguez, B.M., Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.C., Cámara, M., Díez-Marqués, C., Pardo-de-Santayana, M., Molina, M. and Tardío, J. (2011). Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. *Food Res Int*. 1244-1253.

- Salvatore S, Pellegrini, N., Brenna, O.V., Del Rio, D., Frasca, G., Brighenti, F., Tumino, R. (2005). Antioxidant characterization of some Sicilian edible wild greens. *Journal of Agricultural and Food Chemistry*. 53(24): 9465-9471.
- Sánchez-Mata, M.C., Cabrera-Loera, R.D., Morales, P., Fernández-Ruiz, V., Cámara, M., Díez-Marqués, C., Pardo-de-Santayana, M. and Tardío, J. (2012). Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds. *Genetic Resources and Crop Evolution*. 59(3):431-443.
- Scalfi, L., Fogliano, V., Pentangelo, A., Graziani, G., Giordano, I. and Ritieni, A. (2000). Antioxidant activity and general fruit characteristics in different ecotypes of corbarini small tomatoes. *Journal of Agricultural and Food Chemistry*. 48: 1363-1366.
- Scherr, S.J., Jeffrey, A. and McNeely, J.A. (2008). Biodiversity conservation and agricultural sustainability: towards a new paradigm of 'ecoagriculture' landscapes. *Phil. Trans. R. Soc. B*. 363: 477-494.
- Serrano, M., Díaz-Mula, H.M., Zapata, P.J., Castillo, S., Guillén, F., Martínez-Romero, D., Valverde, J.M. and 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.
- Servili M., Selvaggini R., Esposto S., Taticchi A., Montedoro G.F. and Morozzi G., (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A*. 1054: 113-127.
- Silalahi, J. (2002). Anticancer and health protective properties of citrus fruit components. *Asia Pacific Journal of Clinical Nutrition*. 11: 79-84.
- Simopoulos, A.P. (2004). Omega-3 Fatty Acids and Antioxidants in Edible Wild Plants. *Biol Res*. 37: 263-277.
- Simopoulos, A.P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty-acids: evolutionary aspects. En: Simopoulos AP, Cleland LG (ed.) *Omega-6/omega-3 essential fatty-acids: the scientific evidence*. World Review of Nutrition and Dietetics, vol. 92. Karger, Basel, pp 1-22.
- Soffer, T. and Mannheim, C.H. (1994). Optimization of enzymatic peeling of oranges and pomelo. *Lebensmittel Wissen Und Technology*. 27: 245-248.
- Steinmetz KA, Potter JD (1996). Vegetables, Fruit, and Cancer Prevention: A Review. *J. Am. Diet. Assoc*. 96: 1027-1039.
- Strategic Plan for Biodiversity 2011-2020. <https://www.cbd.int/undb>. Accessed April 2014.
- Suutarinen, M., Mustranta, A., Autio, K., Salmenkallio-Marttila, M., Ahvenainen, R. and Buchert, J. (2003). The potential of enzymatic peeling of vegetables. *Journal of the Science of Food and Agriculture*. 83: 1556-1564.
- Tanaka, T. (1929). Sur l'origine et la délimitation de l'espèce cher les Citrus. *Ev. Int. Bot. Appl. D'Agric. Tropicale*. 9: 723-725.
- Tardío, J. (2011). Alimentos silvestres: la despensa más natural y nutritiva. *Ambienta*. 95:36-49.

- Tardío, J., Molina, M., Aceituno-Mata, L., Pardo-De-Santayana, M., Morales, R., Fernández-Ruiz, V., Morales, P., García, P., Cámara, M. and Sánchez-Mata, M. C. (2011). *Montia fontana* L. (*Portulacaceae*), an interesting wild vegetable traditionally consumed in the Iberian Peninsula. *Genetic Resources and Crop Evolution*. 58: 1105-1118.
- Tardío, J. (2010). Spring is coming: the gathering and consumption of wild vegetables in Spain". En: Pardo de Santayana M, Pieroni A, Puri R (ed.) *Ethnobotany in the New Europe: people, health and wild plant resources*. Berghahn Books, Oxford-New York, pp 211-238.
- Tardío, J., Pardo de Santayana, M. and Morales, R. (2006). Ethnobotanical review of wild edible plants in Spain. *Bot J Linn Soc*. 152(1): 27-72.
- Tardío J, Pascual, H. and Morales, R. (2005). Wild food plants traditionally used in the province of Madrid". *Econ Bot*. 59(2): 122-136.
- Tardío, J., Pascual, H. and Morales, R. (2002). *Alimentos silvestres de Madrid. Guía de plantas y setas de uso alimentario tradicional en la Comunidad de Madrid*. Ediciones La Librería, Madrid.
- Ting, S..V. and Rouseff, R.L. (1986). *Citrus fruits and their products. Analysis and Technology*. NY: Marcel Dekker.
- Toledo, A., Burlingame, B. (2006). Biodiversity and nutrition: A common path toward global food security and sustainable development. *Journal of Food Composition and Analysis*. 19: 477-483.
- Turan M., Kordali, S., Zengin, H., Dursun, A. and Sezen, Y. (2003). Macro and micro mineral content of some wild edible leaves consumed in eas-tern Anatolia. *Soil Plant Sci*. 53: 129- 137.
- Ullah, J., Hamayoun, M., Ahmad, T., Ayub, M. and Zarafullah, M. (2003). Effect of Light, Natural and Synthetic Antioxidants on Stability of Edible Oil and Fats. *Asian J. Plant Sci*. 2(17-24): 1192-1194.
- UNESCO (2010). <http://www.unesco.org/new/es/education/themes/leading-the-international-agenda/efareport/reports/2010-marginalization/>. Accessed April 2014.
- Valdés, P., Botella, M.A., Amorós, A., María Serrano, M. and Pretel, M.T. (2005). Aptitudes para el pelado enzimático de diferentes variedades tradicionales de naranja (*Citrus sinensis* (L.) osbeck). *Fruticultura Profesional*. 154: 51-56.
- Verde, A., Rivera, D., Heinrich, M., Fajardo, J., Inocencio, C., Llorach, R., Obón, C. (2003). Plantas alimenticias recolectadas tradicionalmente en la provincia de Albacete y zonas próximas, su uso tradicional en la medicina popular y su potencial como nutracéuticos. *Sabuco Rev Est Albacet*. 4: 35-72.
- Vinson, J.A., Su, X., Zubik, L. and Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry*. 49: 5315-5321.
- Wang, S., Liu J., Feng Y., Niu X., Giovannoni J. and Liu Y. (2008). Altered plastid levels and potential for improved fruit nutrient content by down regulation of the tomato DDB1-interacting protein CUL4. *Plant J*. 55: 89 103.
- Wang, S.Y. and Stretch, A.W. (2001). Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. *Journal of Agricultural and Food Chemistry*, 49, 969-974.

- Whitaker, J.R. (1984). Pectic substances, pectic enzymes and haze formation in fruit juices. *Enzyme and Microbial Technology*. 6: 341-349.
- Wilson, E.O. (1992). *The diversity of life*. Penguin, London. Pp. 275.
- Wojdylo, A., Oszmianski, J. and Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*. 105: 940-949.
- Wolfe, K., Wu, X. and Liu, R.H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*: 51: 609-614.
- Zeghichi, S., Kallithraka, S., Simopoulos, A.P. and Kipriotakis, Z. (2003). Nutritional composition of selected wild plants in the diet of Crete. En: Simopoulos AP, Gopalan C (ed.) *Plants in Human Health and Nutrition Policy*. *World Review of Nutrition and Dietetics*, vol. 91. Karger, Basel, pp 22-40.

Objetives

OBJECTIVES

According to FAO, around 30 000 edible plant species have been identified, from which more than 7 000 have been employed during the history of mankind for satisfying basic needs like food, clothes, fibres, medicines, building materials and fuels. At present, no more than 150 species are commercially cultivated and, from them, 30 species are 90 % of the calorie intake of human diet and only 3 species (rice, wheat and corn) represent more than half of that contribution. During the last decades, the new demands of the consumers of more healthy and varied products, and a higher technological capacity are leading to the domestication and the use of an increasing number of wild plants, both for food and other kinds of uses. These species can be endangered because they are rejected by science and development and because the production areas of crops with better yields that are under a deeper research are growing.

Therefore, in the present Doctoral Thesis the **Main Objective** was:

To know the potential of wild edible plants and traditional fruit species for their use in modern diet as ingredients in functional foods or as minimally processed foods. This could contribute to improving the offer of products for food industry and could favour the cultivation of those species and varieties and, therefore, the conservation of biodiversity.

To fulfil with this global objective the following **Specific Objectives** were:

1. To develop a fresh-cut product from traditional varieties of orange. The obtaining by enzymatic peeling of segments of a traditional variety of orange will be optimised, and its storage under modified atmosphere will be studied through the analysis of the evolution of different parameters of organoleptic, microbiological and nutritional quality.
2. To select the species of wild edible plants (WEP) with interest thanks to their nutritional value or as a potential source of natural antioxidants. Some properties of edible parts of these plants (leaves, flowers, roots, fruits or seeds) will be studied. Their organoleptic characteristics (flavours, aromas and textures that can be appreciated by consumers) and their nutritional and antioxidant quality will be analysed.
3. To develop new minimally processed drinks using WEP as nutritional supplements or antioxidants. Studies on the enrichment of fruit matrices (natural orange juice and kiwi cremogenate) with WEP selected in prior studies according to their nutritional and/or functional properties will be carried out. The acceptance of the new drinks by consumers will be studied, and other parameters of quality like colour will be analysed, as well as the main oxidation parameters of the new drinks like peroxide

index, the content of ascorbic acid (vitamin C) and the total antioxidant capacity (TEAC).

4. To use WEP as potential antioxidants in edible plant oils. The effect of WEP, selected in prior studies due to their high antioxidant capacity, on the oxidation of different edible plant oils under conditions of accelerated oxidation will be studied. The magnitude of the peroxidation will be studied through the analysis of the concentration of malondialdehyde (MDA) since this is a product of lipid peroxidation.

Results and Discussion

Chapter 1

Obtaining and storage of ready-to-use segments from traditional orange obtained by enzymatic peeling

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Obtaining and storage of ready-to-use segments from traditional orange obtained by enzymatic peeling

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Abstract

The aim of this study is to optimize all parameters implied in the process of obtaining orange segments as minimally process product by enzymatic peeling, from the method of obtention to the storage and also, to evaluate two films with different permeability. Enzymatic peeling was carried out by fruit infusion under vacuum conditions in a commercial preparation of pectinases and cellulases (Peelzym II). The best condition to obtain Cadenera segments by enzymatic peeling was 1 ml/L of Peelzym II applied at 53 kPa with three vacuum pulses of two minutes and a subsequent period of 30 minutes in the enzymatic solution at atmospheric pressure. The segments were packaged in two films with different permeability and they were stored during 10 days at 4 °C. Samplings were carried out after 4, 7 and 10 days of storage. The results showed that the composition of CO₂ in the less permeable film (PA 120) was between 0.7 and 1.2 mL/100mL, while in the most permeable one (PA 240) was already 0.2 mL/100mL. Under these conditions, the weight loss was kept in low levels until day 7 of storage, and it undergoes a significant increase after 10 days. Colour (a/b) increases with the peeling process and it remains without significant changes during the whole storage period for both films. The antioxidant capacity slightly decreases after 10 days of storage. The microbial flora is reduced after the enzymatic peeling. Both the sensory and the microbiological quality are kept in adequate levels for consumption during 7 days. From this moment, they lose sweetness and aroma and the microbial development increases. The use of the most permeable film and a maximum of 7 days of storage are recommended to maintain the most quality of the segments.

Key words

Orange, citrus fruit, enzymatic peeling, minimally processed fruits, ready-to-use, film.

INTRODUCTION

The main lines of innovation in the agri-foodstuffs industry are focused on the obtaining of foods that preserve their organoleptic characteristics and the bioactive compounds of plants by the use of storage techniques like controlled or modified atmospheres, among others. The storage with high levels of CO₂ and low levels of O₂ (keeping the sensory and nutritional quality) of fruits and vegetables considerably extends the storage period at low temperatures (Robles-Sánchez et al., 2009). On the other side, the increase in the standard of living of the population has implied a change in the eating habits by increasing the preference of consumers for natural products, without colours or preservatives, with a high quality and easy to prepare. In response to this demand, "ready-to-use" (RTU) or minimally processed products have been developed, that is, fresh fruits and vegetables, washed and cut, ready to be consumed packaged in modified and cold atmospheres that allows a extension of the shelf-life during some days under these conditions (Artes and Artes, 2003). The oranges presents the ideal conditions for being prepared as a RTU since their distribution in segments allows the sectioning of the fruit without modifying the integrity of the vesicular structure (Pretel et al., 1997). At present, the removal of the albedo and of the segment membranes of the citrus fruits is carried out by chemical or mechanical means that imply high water expenditure and a significant negative impact for the environment (Pretel et al., 1998). Enzymatic peeling is an alternative that could reduce the use of water and the degree of contamination in the obtaining of peeled citrus fruits. This new method to obtain peeled citrus fruit is based on infusion under vacuum conditions of an enzymatic preparation, which degrades the pectic substances present in the cell walls (Bruemmer et al., 1978; Pretel et al., 2007a; Pretel et al., 2008). Many parameters must be taken into account in order to obtain good peeling effectiveness, such as type of enzymatic preparation and its concentration (Bruemmer, 1981; Baker et al., 1989; Soffer and Mannheim, 1994), and the vacuum conditions to allow the entrance of enzymatic solution towards the interior of the mesocarp (Pretel et al., 1997; Pretel et al., 2006; Baker and Wicker, 1996; Prakash et al., 2001). Moreover, the effect of the incubation time on peeled fruits quality may be also important (Rohuana and Mannheim, 1994; Soffer and Mannheim, 1994; Pretel et al., 1998; Prakash et al., 2001). The main innovations of this study are both the optimisation of the obtaining of segments by enzymatic peeling in this variety, and the later study of the storage of these segments for their marketing as RTU product.

On the other hand, the market sales of RTU fresh vegetables have grown rapidly in recent decades as a result of changes in consumer attitudes, especially consumption of fresh-cut lettuce and carrot due to their use in prepared salads (Rico et al., 2007). However, the marketing of fruits, in particular orange, as RTU is nearly nonexistent because there are not enough scientific studies regarding their elaboration or preservation. It is well-known that processing of vegetables promotes a faster

physiological deterioration, biochemical changes and microbial degradation of the product even when only slight processing operations are used (O’Beirne and Francis, 2003), which may result in degradation of the colour, texture and flavour (Kabir, 1994; Varoquaux and Wiley, 1994). Reduced O₂ and high CO₂ levels have proven to effectively control enzymatic browning, softening and decay in fresh-cut vegetables (Oms-Oliu et al., 2008), the full beneficial effect of modified atmosphere being attained by correctly choosing the packaging materials (Allende et al., 2006).

This work offers new data about a topic that has not been deeply studied, that is, the obtaining and storage of ready-to-eat citrus fruits by non-contaminant methods (enzymatic peeling). Moreover, this is the first work that globally optimises all parameters implied in the process of obtaining of orange segments as RTU product, from the obtaining of segments by enzymatic peeling until their storage.

MATERIALS AND METHODS

Plant material

A traditional variety of orange fruit (*Citrus sinensis* (L.) Osbeck cv. Cadenera) grown in the South-East of Spain was used. This variety belongs to the group of common oranges that includes white oranges (Rivera et al., 1998). Fruits were harvested at the optimal ripening stage (average respiratory rate 14.53 ± 2.30 mg CO₂ kg⁻¹h⁻¹, ethylene production rate 0.12 ± 0.01 nl g⁻¹h⁻¹, titratable acidity 1.04 ± 0.04 g 100 g⁻¹ and soluble solids content at 20°C 14.3 ± 1.26 °Brix). The acidity and soluble solids were analysed according to Pretel et al. (1998).

Plastic films

The plastic films employed were provided by the firm Sidlaw Packaging P-Plus. Two microperforated polypropylene plastics with non-selective permeability were employed: Film PA120 (Permeability at 25 °C at CO₂ and O₂: 120,000 mL d⁻¹ m²; thickness: 35 µ) and Film PA240 (Permeability at 25 °C at CO₂ and O₂: 240,000 mL d⁻¹ m²; thickness: 35 µm).

Commercial enzyme preparation

The pectolytic enzyme preparation used, provided by the company Novo Nordisk Ferment Ltd (Dittingen, Switzerland), was Peelzym II produced by *Aspergillus niger*. This enzymatic product is very suitable for the degradation of albedo and carpelar membrane due to its high pectinase and polygalacturonase activities (Pretel et al., 2005).

Determination of ideal peeling conditions

Determination of the best combination of vacuum pressure and application time: Potential enzymatic saturation of albedo (PESA) was considered as the amount of enzymatic solution that the albedo fruit would be able to absorb. To evaluate PESA,

oranges without any visible external damage were placed in a water bath at 40°C to reach the recommended temperature for enzymatic peeling (Pretel et al., 1997; Soffer and Manheim, 1994). The entire fruit surface was homogeneously perforated by rolling the oranges over a 1 m² wood plate provided with cylindrical metal parts of 5 mm long and 1 mm diameter. The distance between each cylindrical metal part was of 10 mm. With this system 8 ± 3 perforations per cm² in the albedo fruit were produced. After perforation the fruits were placed in a vacuum tank containing 9 L of the Peelzym II solution (1ml/L) with Chinese ink (1:10) at 40 °C and submitted to different vacuum pressures, 27, 40, 53, 67, 80 and 93 kPa during different application times (pulses of 2 minutes). The PESA was measured using two parameters: percentage of fruit weight increase and percentage of the albedo surface dyed with ink. In all experiments 10 fruits were taken for each vacuum pressure and application time according to Pretel et al. (2007a) From this experiment the best combination of vacuum pressure and application time was selected.

Optimization of the incubation time for enzymatic peeling process: Peelzym II concentration at 1 or 2 ml/L, were used according to the previous experiments. Sixty orange fruits (thirty for each concentration of Peelzym II) without any visible external damage were washed with chlorinated water (300 ppm), placed in a water bath at 40°C and perforated as previously described. Then, orange fruits were exposed to the vacuum conditions previously optimised and thereafter vacuum was released and orange fruits were left to incubate with the enzymatic solution at atmospheric pressure. Samplings of five fruit were undertaken after six different incubation times (10, 20, 30, 40, 50 and 60 min) and the peeling efficiency was evaluated, according to five visual parameters dealing the final product quality. Since these parameters were subjective, each of them was independently assessed by five judges for each of the five fruits of each experiment. The quality parameter evaluated and scores for them were the following (Pretel et al., 2007a):

- a.- Percentage of albedo not attacked: an estimation of the percentage of the fruit surface not attacked by the enzymatic solution.
- b.- Ease of skin removing (albedo + flavedo): from very easy (5) to very difficult (1).
- c.- Fruit firmness: from high (5) to low (1).
- d.- Ease of segment separation: from very easy (5) to very difficult (1).
- e.- Percentage of commercial viable segments: That is, percentage of segments without defects.

Enzymatic Peeling and Sample Preparation: Enzymatic peeling was carried out by vacuum infusion of an enzymatic solution of Peelzym II (1 ml/L) applied at 53 kPa with three vacuum pulses of two minutes, 40°C to remove the peel, and a subsequent period of 30 minutes in the enzymatic solution at atmospheric pressure (these conditions were previously optimized). Fruits were rinsed by immersion in water for 30 min at 4°C. The segments were withdrawn and manually centrifuged to remove water from their surfaces,

and then packaged. Different plastic bags (interchange surface 1000 cm²) were filled with approximately 300 g of oranges and each was fitted with a silicon septum used for sampling the atmosphere for the purpose of analysis. All samples were stored during 10 days at 4±1 °C, the temperature normally used in the commercialization of these products. Variation of the atmosphere composition inside the bags was checked daily. Periodic samplings were carried out on three trays of each film at 4, 7 and 10 days of storage in which weight loss, colour, sensory quality, antioxidant capacity and microbiological and sensory quality of segments were analysed. Some parameters like colour and microbial content were also analysed before peeling (BP) and after peeling (AP) in order to know if, apart from the preservation method, the peeling process has any effect on the quality of segments.

Evaluation of the quality of segments during storage

Gas analysis inside packages: A silicone septum was located on each bag's surface in order to enable sampling gas inside each package. 1 mL of the bag headspace atmosphere was withdrawn using an airtight syringe and the CO₂ and O₂ concentrations were measured by gas chromatography with a GC 14B (Shimadzu, Tokyo, Japan), equipped with a thermal conductivity detector (TCD) and a molecular sieve 5A column, 80-100 mesh (Carbosieve SII. Supelco Inc., Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperature were 50° and 110 °C, respectively. Helium was used as the carrier gas with a flow rate of 50 mL/min. Results are expressed as kPa of O₂ and CO₂ inside the bags and are the mean ± SE of duplicate determinations in each bag (n=5). A further 1-mL of the bag atmosphere was used to quantify ethylene concentration using a Hewlett-Packard gas chromatograph model 5890 (Wilmington, DE, USA) equipped with a flame ionisation detector and a stainless steel column packed with 80/100 mesh-activated alumina. Oven temperature was 90 °C and injector and detector temperatures 150°C. N₂ was used as the carrier gas at a flow rate of 28 mL/min. Results are expressed as µL L⁻¹ inside the bags and are the mean±SE of duplicate determinations in each bag (n=5).

Weight loss and colour: Citrus segment lots were weighted before packaging and after the storage period and weight loss was calculated as percentage of their initial weight (n=10). Colour was determined using a reflection colorimeter Hunter model D-25A-2 with 45° illumination, which expresses colour in coordinates L*, a* and b*. Two measurements were made at opposite sites of the each fruit segment and results represent the mean±SE of the measurements taken from of 10 fruits segment.

Sensory evaluation: Sensory analysis to compare the quality of orange segments was carried out by 10 trained adults, aged to 25-40 (5 female and 5 male). The panel was trained in a pretest in which orange fruit with extremely low or high attributes (aroma, lack of bitterness, chewiness and segment firmness) were evaluated (Pretel et al., 1998). A laboratory of sensory analyses with an individual booth for each panellist was used.

Panellists were randomly served five segments from different films for each variety to evaluate the above mentioned attributes on a ranked scale of 1 to 5. The selection and training of tasters, as well as the fitting-out of the tasting room, were carried out according to UNE (1995).

Determination of Total antioxidant activity (TAA): Total antioxidant activity was determined using the enzymatic system composed of the chromophore 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which ABTS^{•+} radicals are generated. After that the vegetable extracts are added and their antioxidant compounds regenerate the ABTS (Cano et al., 1998; Pretel et al., 2004). The reaction mixture contained 1.5 mM ABTS, 15 μ M hydrogen peroxide and 0.25 μ M HRP in 50 mM glycine-HCl buffer (pH 4.5) in a total volume of 2 ml. The assay temperature was 25 °C and the reaction was monitored at 414 nm until a stable absorbance was obtained using a UNICAM Helios α spectrophotometer (Cambridge, UK). After that, a suitable amount of orange extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with L-ascorbic acid (0-20 nmol) from Sigma (Poole, Dorset, England), and results are expressed as mg of trolox acid equivalent per 100 grams of fresh weight (mg/100 g).

Microbiological analyses: Microbiological growth during the storage of samples was determined by total plate count on Plate Count Agar (PCA, Oxoid), by Lactobacillus count on MRS Agar (Oxoid) and by yeast and mould count on Rose-Bengal Chloranphenicol Agar (Oxoid). Samples were examined on days 3, 7 and 10. Three bags were opened under sterile conditions and a 10 g sample was placed into a stomacher bag with 90 mL of peptone water (Oxoid). Samples were homogenized in peptone water. Appropriate dilutions were plated on to duplicate plates of PCA, MRS or Rose Bengal medium. Plates were incubated at 30 °C for 48-72 and counts were obtained.

Statistical analysis

Data for the physical, chemical and visual quality parameters were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatments. Mean comparisons were performed using the HSD Tukey's test to examine if differences between plastic films or control and storage time were significant at $P < 0.05$. All analyses were performed with a statistical software package SPSS v. 11.0 for Windows. LSDs ($P = 0.05$) were calculated for mean separations and are showed in figures.

RESULTS AND DISCUSSION

Ideal peeling conditions

Different vacuum conditions were applied to orange fruit to determine the minimum vacuum pressure and the minimum number of pulses to be applied to the fruits to allow the entrance of an adequate amount of the enzymatic solution in the albedo and between the segments of the Cadenera orange. A total saturation of the enzymatic solution in the albedo was achieved with pressures between 53 and 93 kPa applied in three vacuum pulses of two minutes, as it can be deduced from the results of the PESA studies, considering as the increase in fresh weight parameter (**Figure 1a**) and the albedo surface dyed with the ink solution (**Figure 1b**). In order to obtain segments it is important to determine the vacuum conditions at which the fruit medule begins to be dyed. This would indicate the penetration of solution inside the fruit and its separation into individual segments (Baker and Bruemmer, 1989; Pretel et al., 2007b). The vacuum pressures with which the medule was dyed began from 53 kPa and three vacuum pulses. Therefore, to obtain segments from Cadenera variety, the best vacuum conditions are 53 kPa with three vacuum pulses. The use of 67 to 93 kPa would not be recommended because an excess of enzyme solution in tissue as porous as the albedo could be a disadvantage for the process of enzymatic peeling, in which the infiltrated material can not be used again (Baker and Wicker, 1996).

On the other hand, it was shown that the effectiveness of the process varied greatly depending on the concentration of the enzyme used (Bruemmer et al., 1978; Pretel et al., 1997; Toker and Bayindirli, 2003). The enzymatic solutions are expensive and it is possible to reduce the amount of enzymatic solution by increasing the incubation time (Prakash et al., 2001). The highest albedo percentage not attacked by the enzyme was found after 10 min of incubation with 1 ml L⁻¹ of Peelzym II (17.7 %±3.2) (**Table1**), which would decrease the quality of the final product (Pretel et al., 2007b). However, after 20 min the percentage of albedo not attacked was less than 3%, a percentage that allows a very good peeling efficiency. The ease of skin separation was reached from 10 minutes of incubation with 2 ml L⁻¹ of enzyme preparation, while with 1ml L⁻¹ the maximum ease of skin was reached after 20 minutes of incubation. The degree of segment separation with 1 ml L⁻¹ of enzyme preparation has its optimum after 30 min incubation time (4.67±0.47) with a percentage of albedo not attacked of 17.7±3.20 %. With 2 ml L⁻¹ and more than 30 min incubation time, the segments separation did not greatly differ. Prakash et al. (2001) found that for the enzymatic peeling of grapefruit a minimum of 12 minutes of incubation was needed to obtain a good ease of peeling, while with incubation times above 30 minutes the segments texture was damaged. Therefore, 30 minutes could be considered as the best time for obtaining peeled segments form Cadenera variety, and the best Peelzym II concentration was 1ml L⁻¹. With 20 minutes of incubation good results are also obtained but increasing the concentration of Peelzym II up to 2 ml L⁻¹.

Table 1: Peeling efficiency of fruits at different incubation times with 1 and 2 ml L⁻¹ of Peelzym II.

Incubation time (min)	Peelzym II (ml L ⁻¹)	*Albedo not attacked (%)	*Ease of skin separation	*Ease of segment separation	**Segment firmness	**Commercial viable segments (%)
10	1	17.7±3.20a	3.7±0.6a	4.67±0.47a	4.0±0.71a	90±6.14a
	2	8.34±2.36a	5±0b	4.12±0.24a	4.17±0.24a	86.67±9.43a
20	1	1.31±1.36b	5±0b	4.84±0.24a	4.0±0.41a	100±5.1a
	2	2.84±1.18b	4.7±0.5b	3.84±0.47b	3.84±0.85a	85.34±7a
30	1	3.34±2.36b	5±0b	4.67±0.47a	4.67±0.24a	100±0a
	2	3.30±0.8b	5±0b	3.84±0.43b	3.67±0.17a	96.67±4.71a
40	1	2.34±2.05b	5±0b	3.50±0.41b	4.01±0.71a	100±3.2a
	2	1.50±1.08b	5±0b	4.0±0.39a	3.34±0.47a	93.34±4.71a
50	1	1.67±2.36b	5±0b	2.67±0.24c	3.17±1.03a	76.67±4.25a
	2	1.67±1.91b	5±0b	4.0±0.41a	3.84±0.85a	66.67±4.71b
60	1	0.92±0.02b	5±0b	3.17±0.24b	3.34±0.24a	73.34±4.43a
	2	0.67±0.94b	5±0b	3.67±0.45b	3.0±0.82a	63.34±3.57b

Data are the mean ± SE of evaluations made independently in five fruits (*) or segments (**) for five judges. Values within a row with similar letters are not significantly different (P<0.05).

Figure 1: Increase in weight of fruits (a) and albedo surface dyed with ink (b) of fruits of the Cadenera variety after applying different vacuum pressures (27, 40, 53, 80 and 93 kPa) for different times: 2 min (2 min), 4 min in two pulses (2+2 min) and 6 min in three pulses (2+2+2 min). Data are the mean of 10 fruits and the LSD_{0,05} value is shown.

Development of the atmosphere in the different films

The evolution of the internal atmosphere of the bags containing the orange segments is shown in **Figure 2**. A passive modification of the atmosphere with an increase of CO₂ (**Figure 2a**) and decrease of O₂ (**Figure 2b**) took place with both films. The composition of the atmosphere in the most impermeable film (PA 120) was around 0.7 mL/100mL of CO₂ and 21 mL/100mL of O₂ during the first seven days, although 1.2 mL/100mL of CO₂ can be achieved after 10 days of storage. In the most permeable film (PA 240) the concentration of CO₂ was 0.2 mL/100mL during the whole experience, and the concentration of O₂ was kept in the same levels than the film PA 120, that is, around 21 mL/100mL. In both films, especially in the most permeable PA 240, the concentrations of CO₂ are low and the O₂ remains in values close to those of the normal atmosphere.

Therefore it cannot be expected the formation of odd flavours or undesirable effects, because they usually appear with higher concentrations of CO₂ and lower of O₂ (Pretel *et al.*, 1998, Del Valle *et al.*, 2009). However, the packaging plays a significant role in order to prevent dehydration and to maintain texture (Brecht, *et al.*, 2003). Moreover, it has been shown in table grape that the atmospheres slightly CO₂ enriched can affect some quality parameters like stopping the weight loss or delaying the microbial development (Pretel *et al.*, 2006). The ethylene levels inside the bags (**Figure 2c**) are very low in both films during the whole storage period (under 0.06 ppm). The slight increase of ethylene observed, especially in the most impermeable film PA 120 after 10 days of storage, could be due to the increase of the microbial development, as other authors have found in pepper stored under modified atmospheres (Ibe and Grogan, 1986) and that has been also found in this work, as it will be later exposed (**Figure 6**).

Figure 2: Evolution of the internal atmosphere (a) mL/100mL CO₂, (b) mL/100mL O₂ and (c) ppm C₂H₄ during 10 days of storage at 4 °C inside the bags made of films PA 120 and PA 240. Each data was represented as the mean ± SE of the measurements made in duplicate in five bags LSD_{0.05} value is shown.

Evolution of the weight loss, colour, antioxidant capacity and sensory quality

Consumers consider the aspect of products as a primary factor of valuation, and texture and colour are the main attributes for considering the freshness of the vegetables (Kays, 1999). The potential of MAP to extend shelf-life for many foods has been well documented (Brecht et al., 2003; Jacxsens et al., 2001; Salveit, 2003). **Figure 3** shows the weight loss of the segments during the storage in modified atmosphere in the two tested films. The weight losses are very low during the whole experience, in values under 0.7%, although they increase as the storage period advances. After a week of packaging of the segments, the weight loss is significantly higher ($p < 0.05$) in the film PA 240 than in PA 120, but the differences in this parameter between both films are not significant neither at the beginning of storage, nor after 4 days nor after 10 days of storage.

Figure 3: Evolution of the weight loss of “Cadenera” orange segments obtained by enzymatic peeling during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five segments from three different trays from each bag. $LSD_{0.05}$ value is shown.

On the other side, the characteristic colour of the segments is defined by a mixture of red (+ a^*) and yellow (+ b^*), so the a^*/b^* coefficient gives an idea of the degree in which the red and yellow colours contribute to the orange colour typical of these fruits due to the accumulation of different carotenoids. There are evidences that show that the ratio between yellow carotenoids and orange carotenoids is a good indicator of the characteristic colour of each variety (Alquézar et al., 2008). Therefore, a high a^*/b^* value should indicate a more intense orange colour, while a low a^*/b^* value should indicate a loss of the characteristic colour. As **Figure 4** shows, changes in colour (parameter a/b) are low during the 10 days of storage, and the differences between the two films employed

are not significant, a fact showing that the storage in modified atmospheres keep the initial colour of the segments, as it has been observed in other orange fruits like mangoes (Pesis et al., 2000). However, after the process of peeling, the segments show significantly higher values ($p < 0.05$) of the parameter a/b (-0.18 ± 0.002) than before peeling (-0.21 ± 0.010). This means that the process of enzymatic peeling increases the orange colour of segments, so they are more attractive for the consumer, probably due to the fact that the enzymes employed during the process remove the rests of albedo and skin that can hide the colour of the segments before the enzymatic peeling.

Figure 4: Evolution of colour (a/b) of “Cadenera” orange segments obtained by enzymatic peeling, before peeling (BP), after peeling (AP) and during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five segments from three different trays from each bag $LSD_{0.05}$ value is shown.

The total antioxidant activity (TAA) of vegetables depends on many bioactive phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments (Oms-Oliu et al., 2008). It has been showed that the use of modified atmospheres is a good method for preserving the antioxidant capacity in vegetables (Murcia et al., 2009). Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported (Cortés et al., 2008). The Total antioxidant activity (TAA) of the segments during the storage in atmosphere slightly enriched with CO_2 in the two tested films is shown in **Figure 5**. The antioxidant capacity did not change after the enzymatic peeling because it changed from 53.6 ± 0.6 before peeling to 52.8 ± 1.4 mg Trolox/199g FW after the enzymatic process. The storage of orange segments in different atmospheres

slightly enriched with CO₂ did not affect the antioxidant capacity during the first week, although after 10 days of storage it significantly decreases ($p < 0.005$) reaching values of 46.7 ± 0.5 in the less permeable film, probably due to a degradation of C vitamin (Villanueva et al., 2005). Other studies have shown that the antioxidant capacity of juices treated with HHP (high hydrostatic pressure) and cold stored at 4 ± 2 °C during 21 days did not change (Fernández-García et al., 2001). In general, most authors coincide with our results, and the antioxidant capacity does not change during storage (Alasalvar et al., 2005; Murcia et al., 2009; Robles-Sanchez et al., 2009), although sometimes the decrease of C vitamin could explain the decrease of the antioxidant capacity of some vegetables stored under modified atmosphere (Villanueva et al., 2005).

Figure 5: Evolution of the antioxidant capacity of “Cadenera” orange segments obtained by enzymatic peeling, before peeling (BP), after peeling (AP) and during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five segments from three different trays from each bag. LSD_{0.05} value is shown.

Table 2 shows the results of the sensory analysis. It can be observed that the segments, in both films, are kept in optimum conditions during the first 4 days. Although the differences are not statistically significant ($p < 0.05\%$), after a week of storage, the segments stored in the most impermeable film PA 120 loss sweetness and aroma, while the segments stored in the most permeable film PA 240 only shows a decrease in aroma and not in sweetness. These results state that the enzymatically peeled orange segments can be stored in atmospheres slightly enriched in CO₂ (between 0.2 mL/100mL and 0.7 mL/100mL) during a week without loss of sensory quality, because the atmospheres with

high levels of CO₂ cause higher losses of aroma and flavour, as it can be shown in manually peeled orange segments under modified atmospheres (Pretel et al., 2008).

Table 2: Evolution of the sensory analysis (sweetness, aroma, chewiness and firmness) of “Cadenera” orange segments obtained by enzymatic peeling during 10 days of storage at 4 °C in the films PA 120 and PA 240.

Film	Parameter	Day 0	Day 4	Day 7
PA 120	Sweetness	3.08 ± 0.76a	3.54±0.62a	2.52±0.31a
	Aroma	2.92± 0.49a	2.71±0.31a	1.51±0.22b
	Chewiness	4.31±0.85a	4.40±0.14a	4.92±0.32a
	Firmness	2.35±0.69a	2.92±0.81a	4.84±0.33a
PA 240	Sweetness	3.08 ± 0.76a	3.05±0.04a	3.02±0.21a
	Aroma	2.92± 0.49a	3.28±0.62a	2.21±0.14b
	Chewiness	4.31±0.85a	4.37±0.11a	4.8±0.51a
	Firmness	2.35±0.69a	3.06±1.24a	4.9±0.21a

Data are the mean ± SE of determinations made in five segments for five judges from different films. Values within a row with similar letters are not significantly different (P<0.05).

Influence of the enzymatic peeling and the film permeability on the microbial development

The use of low temperatures is one of the main microbiological control methods, but some authors have mentioned that CO₂ rich and O₂ poor atmospheres also delay the microbial development (Carlin et al., 1990), although some authors do not observe that inhibition (Berrang et al., 1990). It is interesting to mention the decrease of the microbial development (**Figure 6**), specially of moulds and yeasts (**Figure 6b**) after the peeling process, probably due to the fact that the enzymes employed in the peeling also have a fungicide or bactericide effect (Pretel et al., 1998; Pinnavaia et al., 2006). However, it is possible that the enzyme peeling removes excess peel, which may harbor bacteria and this may explain the decrease in bacterial count after peeling. The mesophilic aerobic microflora (figure 6a) remains in acceptable levels close to the initial levels up to day 7 of storage with few differences between both films, although they are always slightly higher in the most permeable film. After a week of storage, the development of mesophilic flora experiences a considerable increase so the segments are not commercially viable. This can be due to the fact that the CO₂ concentration in the internal atmosphere of the bags is not high enough for stopping the development of the mesophilic flora, as it has been observed in manually separated orange segments with CO₂ concentrations over 5 mL/100mL (Pretel et al., 1998). The evolution of the content of moulds and yeasts (figure 6b) shows a similar

behaviour, with levels close to the initial ones up to day 4 in the most impermeable film. The CO₂ concentrations reached inside the bags (0.7 mL/100mL) can probably have a fungistat effect, as it has been suggested by some authors (Carlin et al., 1990). From day 7 in the film PA 120 and from day 4 in the film PA 240, the levels of moulds and yeasts increase. The lactic microflora (figure 6c) remains close to the initial values during the first week of storage in both films and it considerably increases after 10 days. The differences between the films are not significant. However, one of the main concerns about the use of treatments for extending the shelf life of RTU products associated to the use of high levels of CO₂ and low levels of O₂ is that these treatments can create favourable environments for the development of pathogenic bacteria (Rosnes et al., 2003; Rico et al., 2007).

Figure 6: Evolution of (A) mesophilic aerobic microflora lg CFU/g, (B) moulds and yeasts lg CFU/g and (C) lactic microflora lg CFU/g before peeling (BP), after peeling (AP) and during 10 days of storage at 4 °C in the films PA 120 and PA 2. a and b represent the homogeneous sub-groups generated by Tukey's test, using the film as factor, at the 95% confidence level ($p \leq 0.05$).

CONCLUSIONS

The best condition to obtain Cadenera segments by enzymatic peeling was 1 ml/L of Peelzym II applied at 53 kPa with three vacuum pulses of two minutes and a subsequent period of 30 minutes in the enzymatic solution at atmospheric pressure. The enzymatic peeling does not affect the antioxidant capacity of the segments, reduces the microbial flora and increases the orange colour. The segments packaged in atmospheres slightly enriched with CO₂ (0.2 in the film PA 240 and 0.7 mL/100mL in the film PA 120) have a shelf life of 7 days without losing their organoleptic and microbiological quality and their antioxidant potential. After 10 days of storage, and especially in the most impermeable film (PA 120) in which levels of CO₂ of 1, 17±0.31mL/100mL are achieved, the antioxidant capacity of the segments slightly but significantly decreases, the weight of loss increases, the microbial contamination increases and there is a loss of sweetness and aroma.

REFERENCES

- Alasalvar C., Al-Farsi M., Quantick P.C., Shahidi F. and Wiktorowicz R. (2005). Effect of chillid storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chemistry* 89: 69-76.
- Allende A., Tomás-Barberán F.A. and Gil I. (2006). Minimal processing for healthy traditional foods. *Trends Food Sci. Technol* 17: 513-519.
- Alquézar B., Rodrigo M.J. and Zacarias L. (2008). Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry* 69: 1997-2007.
- Artés F., and Artés F. (2003) In: Lobo G, González M *Productos Hortofrutícolas Mínimamente Procesados*. Consejería de Agricultura, Ganadería, Pesca y Alimentación, Canarias
- Baker R.A. and Wicker L. (1996). Current and potencial applications of enzyme infusion in the food industry. *Trends in Food Science and Technology* 7: 279-284.
- Baker, R.A. and Bruemmer, J.H., (1989). Quality and stability of enzymatically peeled and sectioned citrus fruit. In J.J. Jen (Ed.) *Quality Factors of Fruits and Vegetables*. American Chemical Society, Washington, DC., pp.140-148.
- Baker, R.A. and Bruemmer, J.H., (1989). Quality and stability of enzymatically peeled and sectioned citrus fruit. In J.J. Jen (Ed.) *Quality Factors of Fruits and Vegetables*. American Chemical Society, Washington, DC., pp.140-148.
- Berrang M.E., Brackett R.E. and Beuchat L.R. (1990). Microbial, color and textural qualities of fresh asparagus, broccoli and cauliflower stored under controlled atmosphere. *Journal of Food Protection* 53: 391-396.
- Brecht J.K., Chau K.V., Fonseca S.C. and Oliviera F.A.R. (2003). CA transport of fresh produce in MAP: designing systems for optimal atmosphere conditions throughout the postharvest handling chain. *Acta Horticulturae*, 600: 799-801.

- Bruemmer J.H. (1981) Method of preparing Citrus fruit. Sections with fresh fruit flavor and appearance. US Patent Number 4.284.651
- Bruemmer J.H., Griffin A.W. and Onayami O. (1978). Sectionizing grapefruit by enzyme digestion. Proceedings Florida State Horticultural Society 91:112-114.
- Cano A., Hernández-Ruiz J., García-Cánovas F., Acosta M. and Arnao M.B. (1998). An end-point method for estimation of the total antioxidant activity in plant material. *Phytochemical Analysis* 9: 196-202.
- Carlin F., Nguyen-The C. and Chambroy Y. (1990). Effects of controlled atmospheres on microbial spoilage, electrolyte leakage and sugar content of fresh 'ready to eat' grated carrots. *International Journal of Food Science and Technology* 25: 110-119.
- Cortés C., Esteve M.J. and Frígola A. (2008). Effect of refrigerated storage on ascorbic acid content of orange juice treated by pulsed electric fields and thermal pasteurization. *European Food Research and Technology* 227: 629-635.
- Del-Valle V., Hernandez-Munoz P., Catala R. and Gavara R. (2009). Optimization of an equilibrium modified atmosphere packaging (EMAP) for minimally processed mandarin segments. *Journal of Food Engineering*. 91: 474-481.
- Fernández García A., Butz P. and Tauscher B. (2001). Effects of high-pressure processing on carotenoid extractability, antioxidant activity, glucose diffusion, and water binding of tomato puree (*Lycopersicon sculentum* Mill). *Journal of Food Science* 66:1033-1038.
- Ibe S.N. and Grogan R.C. (1986). Effect of chilling carbon dioxide and ethylene inhibitors on ethylene production and infection of green bell peppers (*Capsicum-annuum* by *Erwinia-carotovora* ssp *carotovora*. *Biologia Africana* 2: 24-32.
- Jacxsens L., Devlieghere F. and Debevere J. (2001). Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology* 71: 197-210.
- Kabir H. (1994). Fresh-cut vegetables. In: Brods L, Herndon VA (ed). *Modified atmosphere food packaging*. Institute of Packaging Professionals. pp. 155-160.
- Kays S.J. (1999). Preharvest factors affecting appearance. *Postharvest Biology and Technology* 15: 233-247.
- Murcia M.A., Jiménez-Monreal A.M., García-Díaz L., Carmona M., Maggi L. and Martínez-Tomé M. (2009). Antioxidant activity of minimally processed (in modified atmospheres), dehydrated and ready-to-eat- vegetables. *Food and Chemical Toxicology* 47: 2103-2110.
- O'Beirne, D. and Francis G.A., (2003). Reducing the pathogen risk in MAP-prepared produce. In: Ahvenainen (ed.), *Novel food packaging techniques*. Cambridge, UK; Boca Raton, FL: Woodhead Publishing Limited/CRC Press LLC, pp. 231-286.
- Oms-Oliu G., Soliva-Fortuny R. and Martín-Belloso O. (2008). Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research and Technology* 225: 301-311.
- Pesis E., Aarón D., Aharon Z., Ben-Aire R., Aarón N. and Fuchs Y. (2000). Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit. *Postharvest Biology and Technology* 19: 93-101.

- Pinnavaia S., Baldwin E.A., Plotto A., Narciso J. and Senesi E. (2006). Enzyme-peeling of Valencia oranges for fresh-cut slices. *Proceedings of the Florida State Horticultural Society* 119: 335-339.
- Prakash S., Singhal R.S. and Kulkarni P.R. (2001). Enzymic peeling of Indian grapefruit (*Citrus paradisi*). *Journal of the Science of Food and Agriculture* 81:1440-1442.
- Pretel M.T., Amorós A., Botella M.A., Serrano M. and Romojaro F. (2005). Study of albedo and carpelar membrane degradation for further application in enzymatic peeling of citrus fruits. *Journal of the Science of Food and agriculture* 84: 86-90.
- Pretel M.T., Botella M.A., Amorós A., Serrano M., Egea I. and Romojaro F. (2007b). Obtaining fruit segments from a traditional orange variety (*Citrus sinensis* (L.). Osbeck cv. Sangrina) by enzymatic peeling. *European Food Research and Technology* 225: 783-788.
- Pretel M.T., Botella M.A., Amorós A., Zapata P.J. and Serrano M. (2007a) Optimization of vacuum Infusion and incubation time for enzymatic peeling of 'Thomson' and 'Mollar' oranges. *LWT-Food Science and Technology* 40:12-30
- Pretel M.T., Botella M.A., Zapata P. J., Amorós A. and Serrano M. (2004). Antioxidant activity and general fruit characteristics in different traditional orange (*Citrus sinensis* (L.) Osbeck) varieties. *European Food Research and Technoogy* 219: 474-478.
- Pretel M.T., Fernández P.S., Martínez A. and Romojaro F (1998). The effect of modified atmosphere packaging on "ready-to-eat" oranges. *Lebensmittel Wissenchard Und Technology* 31:322-328
- Pretel M.T., Lozano P., Riquelme F. and Romojaro F (1997). Pectic enzymes in fresh fruit processing: optimization of enzymic peeling of oranges. *Process Biochemistry* 32: 43-49
- Pretel M.T., Martínez-Madrid M.C., Martínez J.R., Carreño, J.C. and Romojaro F. (2006). Prolonged storage of 'Aledo' table grapes in a slightly CO₂ enriched atmosphere in combination with generators of SO₂. *LWT-Food Science and Technology* 39: 1109-1116.
- Pretel, M.T., Sanchez-Bel, P., Egea, I. and Romojaro, F., (2008). Enzymatic peeling of citrus fruits: factors affecting degradation of the albedo. In J.A Teixeira da Silva (Ed.), *Tree and forestry Science and Biotechnology Global Science Books*, Islework, UK, pp. 52-59.
- Rico D., Martín-Diana A.B., Barat J.M. and Barry-Ryan C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology* 18:373-386.
- Rivera D., Obón C., Ríos S., Selma C., Méndez F., Verde A. and Cano F. (1998). Las variedades tradicionales de frutales de la cuenca del río Segura. *Catálogo etnobotánico. Cítricos, frutos carnosos y vides*, Murcia.
- Robles-Sánchez R.M., Rojas-Graü M.A., Odriozola-serrano I., González-Aguilar, G.A. and Martín-Belloso O. (2009) Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut 'Kent' mango (*Mangifera indica* L.). *Postharvest Biology and Technology* 51: 384-390
- Rosnes, J.T., Sivertsvik, M. and Skåra, T., (2003). Combining MAP with other preservation techniques. In: R. Ahvenainen (Ed.), *Novel food packaging techniques*. Cambridge, England: Wood-head publishing ltd.

- Rouhana A. and Mannheim C.H. (1994). Optimization of enzymatic peeling of grapefruit. *Lebensmittel Wissenchard Und Technology* 27: 103-107.
- Salveit M.E. (2003). Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology* 27: 3-13.
- Soffer T. and Mannheim C.H. (1994). Optimization of enzymatic peeling of oranges and pomelo. *Lebensmittel Wissenchard Und Technology* 27:245-248.
- Toker I. and Bayindirli A. (2003). Enzymatic peeling of apricots, nectarines and peaches. *Lebensmittel-Wissenschaft und-Technologie* 36 (2): 215-221.
- UNE 87-004-79 (1995). Análisis sensorial. Guía para la instalación de una sala de cata. Asociación Española de Normalización y Certificación, Madrid, España.
- Varoquaux, P. and Wiley, R. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables* New York, USA: Chapman and Hall, pp. 226-268.
- Villanueva M.J., Tenorio M.D., Sagardoy M., Redondo A. and Saco M.D. (2005). Physical, chemical, histological and microbiological changes in fresh green asparagus (*Asparagus officinalis*, L.) stored in modified atmosphere packaging. *Food Chemistry* 91: 609-619.

Chapter 2

Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet

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Abstract

Thirteen species of wild edible plants belonging to eleven botanical families consumed in the traditional Mediterranean diet were evaluated. *Sanguisorba minor*, *Quercus ballota*, and *Sedum sediforme* showed the highest hydrophilic total antioxidant activity (H-TAA) and total phenols. *Asparagus acutifolius*, *Allium ampeloprasum*, *Foeniculum vulgare*, and *Malva sylvestris* presented high levels of potassium, *Malva* and *Asparagus* are interesting due to their zinc content, and *Urtica urens* contains a high content of calcium. Sensory analysis indicated that fruits from *Q. ballota* could be considered very sweet and plants of *Crithmum maritimum* and *Oxalis pes-caprae* very acidic. Moreover, testers highlighted the salty taste of *C. maritimum*, *Mesembryanthemum nodiflorum*, and *Mesembryanthemum cristalinum*, the spicy taste of *A. ampeloprasum*, and the aroma of *F. vulgare*. Our results indicate that increased consumption of the investigated plant species could provide health benefits. Moreover, due to their sensorial properties, they could be used as new ingredients to improve the diversity in modern diet and highly creative cuisine.

Keywords Antioxidant activity, nutritional properties, wild edible plants, modern diet.

INTRODUCTION

The world is presently over dependent on a few plant species. Diversification of production and consumption habits to include a broader range of plant species, particularly those currently identified as underutilised, could significantly contribute to improve health and nutrition, livelihoods, and ecological sustainability. Wild plants have played an important role in supplementing staple foods by supplying trace elements, vitamins, and minerals in order to obtain a balanced diet, and they may do so again in the future. Their interest as a source of nutraceuticals has been highlighted in recent studies (Leonti, 2012). Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants reduces the risk of some cancers, heart and degenerative diseases. Nowadays, consumers appreciate substituting synthetic additives by natural additives, so the natural oxidants present in wild plants could be a possible alternative for agro-food industries, as well as for gastronomy.

Moreover, some organic acids may have biological activity. Tartaric, malic, citric, and succinic acids have shown health benefits as antioxidants due to their ability to chelate metals (Serrano et al., 2009). However, it is well known that oxalic acid has a negative effect on the reduction of the availability of dietary Ca and on the formation of kidney calculi (Guil et al., 1997). There is little published literature available about the comprehensive mineral and nutrient content of many wild plants. Such information is valuable not only for ascertaining the nutrient contents that may be used to supplement the nutritional value of the normal diet, but also for environmentalists involved in the design of conservation programs aimed at protecting and propagating nutritionally valuable wild plant resources. Moreover, some modern cultures still consume wild plants as a normal food source from which fairly high amounts of several nutrients are obtained, and it is widely accepted that leafy green vegetables are significant nutritional sources of minerals (Rivera et al., 2006; Tardío et al., 2006; Łuczaj et al., 2012).

At present, people in cities are accustomed to always consuming the same products cooked the same way, thus they are looking for new products in their meals. They need new colours, flavours, textures, and ingredients (Mlcek and Rop, 2011); and therefore, many restaurant chefs are interested in finding and using plants that have been traditionally employed (Łuczaj et al., 2012). In this way, the study of many plant species that have been part of the Mediterranean diet is important, as they can be an interesting genetic resource for the development of new food products (Rivera et al., 2006; Tardío et al., 2006). Wild edible plants may have great potential as a source of unusual colours and flavours, bioactive compounds, and as sources of dietary supplements or functional foods (Salvatore et al., 2005). In this regard, there is a great variety of wild non-cultivated plants that have been employed in the diet and traditional medicine of the Mediterranean region (Pardo de Santayana et al., 2005), but their use has now decreased, and even disappeared, as a consequence of the industrial revolution.

In Spain and other European countries, wild edible plants have often been consumed, mostly as green vegetables. *Allium ampeloprasum*, *Asparagus acutifolius*, *Foeniculum vulgare*, *Malva sylvestris*, *Sanguisorba minor*, and *Urtica urens* have been part of many traditional dishes, mainly in omelettes or scrambled eggs (Rivera et al., 2006, Tardío et al., 2006). Other species have been eaten raw as a snack: *Oxalis pes-caprae* (peduncle of inflorescences) and *Malva sylvestris* (immature fruits) because of their acidic and sweet tastes, respectively. According to Hedrick (1972), the entire *Crithmum maritimum* plant is "of a spicy taste with a certain saltiness", and has long been held in great repute as an ingredient in salads. It has been cultivated in English gardens and in France, but is more often collected from the shores. *Mesembryanthemum nodiflorum* and *M. cristalinum* have been consumed in some parts of the world. They are extremely tender, slightly salty, and sour. Nowadays, some cooks at Spanish restaurants are using their leaves, cut into small pieces, as ingredients in salads. Acorns from *Quercus ballota* (there is a variety with sweet fruits) have been traditionally consumed in Spain; they are very nutritious due to their high carbohydrate content and can be used roasted and casseroled, added to lentils or in cakes together with honey (Rivera et al., 2006). *Cistus albidus* petals have been eaten as a snack in the countryside and they have a nice pink colour that could be used to add colour to salads. Most of these plant species are good candidates as functional ingredients and could be included in the modern diet.

However, for these wild plants to become an alternative source for the food industry, broader knowledge about their physico-chemical, antioxidant, and nutritional properties is necessary (Martins et al., 2011). These studies could be utilised by gastronomic companies to learn about the agro-industrial potential and promote their exploitation as additives or natural ingredients. It is generally acknowledged that studies on traditional knowledge systems should provide feedback to local communities and society in general (Rivera et al., 2006; Leonti, 2012).

The aim of this paper was to document the antioxidant properties, mineral composition, organic acids content, and sensorial characteristics of different wild plant species traditionally used in Mediterranean areas and/or by different cooks in contemporary cuisine.

MATERIALS AND METHODS

Plant material

Different parts of 13 wild edible plants (Table 1) consumed in the Mediterranean diet were chosen according to previous ethnobotanical studies (Rivera et al., 2006; Tardío et al., 2006) and/or that they are presently used in European restaurants. These samples were gathered in southeast Spain from December 2009 to February 2010. Each species was collected at the moment they were suited for consumption according to their traditional

use, and analyses were carried out on three samples of at least 100 g of the edible portions from 20 plants. Voucher specimens were deposited in the Miguel Hernandez University of Elche Herbarium (UMH). The assignment of species within families, the scientific nomenclature, and the authority attribution for each taxon follow *The Plant List* (2012). The samples were lyophilised after collection, identified, and kept in optimum conditions (-80 °C and protected from light) for subsequent use. The experimental results were expressed as mean $\pm$ standard error (SE) of three replicates.

Antioxidant activity and total phenolics

Total antioxidant activity (TAA) was quantified according to Serrano et al. (2009), which enables the determination of TAA due to both hydrophilic and lipophilic compounds in the same extraction. The TAA (L-TAA and H-TAA) was determined using the enzymatic system composed by the chromophore 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horseradish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide, H_2O_2), in which ABTS $^{+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the extract was proportional to the TAA in the sample. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0-20 μ mol) from Sigma (Madrid, Spain), and the results are expressed as mg of Trolox equivalent 100 g⁻¹.

Total phenolic compounds were quantified according to the method described by Singleton et al. (1999) using the Folin-Ciocalteu Reagent and the results are expressed as mg of gallic acid equivalent 100 g⁻¹.

Hydroxyl radical (OH $^{\bullet}$) and peroxy radical (H_2O_2) scavenging

Samples were prepared according to Egea, Sánchez-Bel, Romojaro & Pretel (2010). The lyophilised material (0.2 g) was extracted for 1 h by stirring at 4 °C in darkness with 40 mL of phosphate buffer (10 mM KH_2PO_4 -KOH buffer, pH 7.4), and centrifuged at 4500 x g for 30 min. Aliquots of the supernatants were used as fruit extract in the different antioxidant assays.

Hydroxyl radical (OH $^{\bullet}$) scavenging potential assay: In a final volume of 1.2 mL, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4 -KOH buffer (pH 7.4), 2.8 mM H_2O_2 , 2.8 mM deoxyribose (DR), 50 μ M $FeCl_3$ pre-mixed with 100 μ M EDTA before addition to the reaction mixture, and 100 μ L of sample. Ascorbate (100 μ M) was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the hydroxyl radical (OH $^{\bullet}$) attack on sugar were measured as described in Egea et al. (2010) at 532 nm. The results are expressed as percentage inhibition of the DR attack, where 100% attack is defined as the absorbance levels recorded for DR without the addition of the samples. The same reaction without ASC was used to determine whether plants could reduce hydroxyl radical generation by avoiding the oxidation of ascorbate rather than by directly scavenging hydroxyl radicals.

Table 1 Plant species analysed.

Species	Family	Plant part analysed	English name	Traditional use
<i>Allium ampeloprasum</i> L.	<i>Amaryllidaceae</i>	Leaves	Great-headed garlic. Levant Garlic. Wild leek	Salad
<i>Asparagus acutifolius</i> L.	<i>Asparagaceae</i>	Young shoots	Wild asparagus	Stew
<i>Cistus albidus</i> L.	<i>Cistaceae</i>	Petals	Rock rose	Snack, salad
<i>Crithmum maritimum</i> L.	<i>Apiaceae</i>	Leaves	Sea fennel, samphire, or rock samphire	Picked in vinegar
<i>Foeniculum vulgare</i> Mill.	<i>Apiaceae</i>	Leaves, young shoots	Fennel	Snack, salad, stew
<i>Malva sylvestris</i> L.	<i>Malvaceae</i>	Leaves	High mallow, Marsh mallow	Stew
<i>Malva sylvestris</i> L.	<i>Malvaceae</i>	Immature fruits	Cheeses	Snack, salad
<i>Mesembryanthemum nodiflorum</i> L.	<i>Aizoaceae</i>	Leaves	Slender-leaf ice plant	Salad
<i>Mesembryanthemum cristalinum</i> L.	<i>Aizoaceae</i>	Leaves	Crystalline ice plant, ice plant	Salad
<i>Oxalis pes-caprae</i> L.	<i>Oxalidaceae</i>	Leaves and peduncle of inflorescence	Cape sorrel	Salad
<i>Quercus ballota</i> Desf.	<i>Fagaceae</i>	Fruits (acorns)	Ballota, belloot or belote oak. Holly oak	Raw, boiled, roasted
<i>Sanguisorba minor</i> Scop.	<i>Rosaceae</i>	Leaves	Burnet	Salad
<i>Sedum sediforme</i> Pau	<i>Crassulaceae</i>	Leaves	Pale stonecrop, white stonecrop	Picked in vinegar
<i>Urtica urens</i> L.	<i>Urticaceae</i>	Tender leaves and shoots	Nettle	Stew

Peroxyl radical (H_2O_2) scavenging potential assay: An aliquot of the sample (100 μL) was incubated with 0.84 mM H_2O_2 for 10 min at 25 $^\circ\text{C}$. Aliquots of these mixtures were then taken and assayed for the remaining H_2O_2 by using the peroxidase system described by Murcia et al. (2007). The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 mL, 0.15 M KH_2PO_4 KOH buffer, pH 7.4, 0.5 mM guayacol, and 10 μL of Sigma type IV horseradish peroxidase. The results are expressed as percentage of disappearance of the H_2O_2 from the reaction medium.

Extraction and quantification of organic acids

The method described by Serrano et al. (2005), with some modifications, was employed. For the extraction, lyophilised material (0.1 g) was homogenated with 10 mL of deionised water at 50 $^\circ\text{C}$ for 3 min. The final solution was then centrifuged at 24000 $\times g$ for 30 min at room temperature. Subsequently, the extract was purified through Durapore 0.45 μm HV membrane filters (Millipore Corporation, USA). The mobile phase was filtered through MFTM 0.45 μm HA membrane filters (Millipore Corporation, USA). Organic acid quantification was carried out in a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min⁻¹. The organic acids were eluted through a Supelco column (Supelcogel C-610H, 30 cm $\times$ 7.8 mm, Supelco Park, Bellefonte, USA), and detected by absorbance at 210 nm. A standard curve of pure organic acids (oxalic, citric, malic, and shikimic) purchased from Sigma (Poole, Dorset, UK) was used for quantification. Results are expressed as mg of acid 100 g⁻¹ (percentage) fresh weight (FW).

Nutrient minerals

Na and K contents were determined in nitric acid (HNO_3): perchloric acid (HClO_4) (2:1 v/v) digestion extract by Jenway PFP7 flame photometry (Bibby Scientific, Dunmow, UK), and Ca and Mg, Fe, and Zn by Unicam Solaar 969 atomic absorption spectrophotometry (Unicam, Cambridge, UK).

Sensory attributes

Ten trained tasters judged 5 sensory attributes in a quantitative manner: sweetness, acidity, bitterness, aroma, and juiciness. To value each attribute, the scale ranged from 0 (minimum value) to 10 (maximum value). The tasters valued the five sensory attributes on raw samples from six species that traditionally have been consumed in salad: *Cistus albidus*, *Malva sylvestris* (immature fruits), *Mesembryanthemum nodiflorum*, *M. cristalinum*, *Oxalis pes-caprae*, and *Sanguisorba minor*. The other species (*Allium ampeloprasum*, *Asparagus acutifolius*, *Foeniculum vulgare*, *Malva sylvestris* (leaves) *Quercus ballota*, *Urtica urens*, *Sedum sediforme*, and *Crithmum maritimum*) traditionally have been consumed after boiling, being part of casseroles or pickled in vinegar, therefore they were evaluated after boiling with tap water in a 1:5 w/v

proportion for 15 min. The tasters also made a global quantitative assessment of each species and qualitatively highlighted the most relevant properties.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were calculated using statistical software (SPSS 14.0, SPSS Inc., Chicago, USA). Correlations between different parameters were computed as Pearson's correlation coefficient (r). Least significant difference values (LSD, $p \leq 0.05$) were used to compare the means.

RESULTS AND DISCUSSION

Antioxidant activity (hydrophilic and lipophilic antioxidant activities) and total phenolic content

The highest H-TAA (**Figure 1a**) was found in *Sanguisorba minor*, *Quercus ballota*, and *Sedum sediforme*, with 1368.91 ± 47.96 , 1186.91 ± 109.31 , and 588.87 ± 35.52 mg of Trolox equivalent 100 g^{-1} FW, respectively. Such high levels of H-TAA are found in some fruits or parts of plants employed for medicinal use (Surveswaran et al., 2007) and in other non-cultivated fruits like blackberry, blueberry, or strawberry trees. This extremely high antioxidant capacity has been attributed to their high phytonutrient content (García-Alonso et al., 2004). Other species studied, like *Cistus albidus* (petals) and *Malva sylvestris* (leaves), also showed a high antioxidant capacity, with values of 132.14 ± 4.91 and 77.92 ± 5.71 mg of Trolox equivalent 100 g^{-1} FW, respectively, although their activity was about twenty times lower than *S. minor* and *Q. ballota* fruits. Dall'Acqua et al. (2008) and Egea et al. (2010) reported that *S. minor* could be considered a possible new antioxidant ingredient for the nutraceutical or functional-food market. The remaining analysed species and immature fruits from *M. sylvestris* showed antioxidant capacity that was about 50 times lower than *S. minor* and *Q. ballota*. Although these activities are not very high, they are similar to grapefruits and oranges, and higher than the antioxidant activity of other fruits like tomato, banana, peach, or apple (Proteggente et al., 2002).

L-TAA (**Figure 1b**) followed a pattern similar to H-TAA (**Figure 1a**), thus plants with the highest L-TAA were again *Q. ballota*, *S. sediforme*, and *S. minor*. However, in the first two species, the L-TAA was 40% and 45% of the total antioxidant activity, while in *S. minor* it was only 10% of the total. No literature is available to contrast the L-TAA results in plants; however, the presence of tocopherol has been related to L-TAA in some fruits (Cho et al., 2007). The high L-TAA has also been related to high levels of carotenoids in sweet cherry cultivars (Serrano et al., 2009).

The main antioxidant substances found in fruits and plants include ascorbic acid, carotenoids and phenolic compounds (Murcia et al., 2004; Egea et al., 2010). The plant

with the highest total phenol levels (530.51 ± 21.12 mg 100 g $^{-1}$ FW) was *S. minor* (**Figure 1c**), whose levels were similar to those found in several cultivars of *Fragaria vesca*, the fruit which is considered to have elevated levels of phenolic compounds (Doumett et al., 2011).

Figure 1: (a) H-TAA, hydrophilic antioxidant activity (mg eq. Trolox 100 g $^{-1}$ FW), (b) L-TAA, lipophilic antioxidant activity (mg eq. Trolox 100 g $^{-1}$ FW) and (c) total phenolic content (mg 100 g $^{-1}$ FW) of different wild edible plants. Data are the mean $\pm$ SE. LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

Consistent with our results, Vanzani et al. (2011) found particularly high levels of polyphenols and ferric reducing antioxidant power in *S. minor*. The *Q. ballota*, *C. albidus*, and *S. sediforme* species also showed high levels of phenolic compounds, 326.91 ± 51.05 , 269.46 ± 21.15 , and 191.53 ± 13.78 mg 100 g⁻¹ FW, respectively. These values are similar and even higher to those found by Proteggente et al. (2002) in regularly consumed fruits considered to have a high polyphenol content, such as strawberry (330 mg 100 g⁻¹ FW), red plum (320 mg 100 g⁻¹ FW) or raspberry (228 mg 100 g⁻¹ FW). Most species in this study presented between 20-80 mg 100 g⁻¹ FW of phenolic compounds; nevertheless, they can also be considered a good natural source of polyphenols. These levels are similar to those found in other fruits like deerberry (Marinova et al., 2005), orange, red cabbage, and broccoli (Proteggente et al., 2002). Finally, the species with the lowest phenol levels was *Oxalis pes-caprae* with 11.56 ± 0.21 mg 100 g⁻¹ FW, below the level found in most regularly consumed fruits (Proteggente et al., 2002).

In this study, a positive relationship was found between H-TAA and total phenolics in most of the species considered according to the equation $y = 2.7x - 83$ with $r^2 = 0.81$. Although not determined in this study, it is well known that ascorbic acid can also contribute to H-TAA (Serrano et al., 2005). However, the activity in the H-TAA assay increases with the number of hydroxyl groups in the antioxidant compound, so antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts under experimental conditions. This relation between total phenols and antioxidant activity has been found in many fruits (Proteggente et al., 2002; Serrano et al., 2009), and suggests that the phenolic compounds could also be mostly responsible for the antioxidant properties of *S. minor* and *Q. ballota*. Recently, it has been reported that the ingestion of certain foods containing high amounts of phenolic compounds has an antioxidant activity *in vivo* by increasing plasmatic antioxidants (Fernández-Panchón et al., 2008).

Hydroxyl radical (OH[•]) and peroxy radical (H₂O₂) scavenging

Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are capable of damaging almost every molecule found in living cells. These radicals have the capacity to join the nucleotides in DNA and cause strand breakage, contributing to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this species is considered one of the quick initiators of the lipid peroxidation process due to abstraction of hydrogen atoms from unsaturated fatty acids (Murcia et al., 2004). **Table 2** shows the inhibition of hydroxyl radicals by extracts of different edible plants. Based on their antioxidant capacity, the species considered in this work can be divided into three groups. In the first are *Allium ampeloprasum*, *Asparagus acutifolius*, *Crithmum maritimum*, *Foeniculum vulgare*, *M. sylvestris* (leaves), *Mesembryanthemum nodiflorum*, *S. minor*, *S. sediforme*, and *Urtica urens*, with inhibition percentages between 40 and 60%. Therefore, according to this assay, they can be considered moderately powerful antioxidants.

Table 2: Scavenging radical OH[•] capacity, and H₂O₂ scavenging of different wild edible plants (% Inhibition) (mean ± SE).

Species	Oxidative attack to DR (% Inhibition) ^a With ASC	Oxidative attack to DR (% Inhibition) ^a Without ASC	Scavenging H ₂ O ₂ (% Inhibition) ^a
<i>Allium ampeloprasum</i> L.	50.46±0.36	29.84±0.68	22.21±6.67
<i>Asparagus acutifolius</i> L.	53.49±1.93	59.79±4.34	62.45±3.42
<i>Cistus albidus</i> L.	31.18±2.56	60.19±1.15	47.48±4.39
<i>Crithmum maritimum</i> L.	52.88±1.71	68.99±11.12	65.30±6.71
<i>Foeniculum vulgare</i> Mill.	46.51±4.69	61.75±7.53	63.55±4.46
<i>Malva sylvestris</i> L. (leaves)	47.16±8.01	81.33±1.67	35.10±6.12
<i>Malva sylvestris</i> L. (immature fruits)	9.90±1.32	65.62±3.52	10.05±3.54
<i>M. nodiflorum</i> L.	42.02±6.5	65.71±1.14	80.49±0.82
<i>M. cristalinum</i> L.	1.38±0.63	70.38±1.08	74.88±2.03
<i>Oxalis pes-caprae</i> L.	30.25±6.34	83.81±4.86	4.09±1.99
<i>Quercus ballota</i> Desf.	31.07±4.41	20.64±3.63	29.9±4.37
<i>Sanguisorba minor</i> Scop.	57.75±9.3	33.5±3.09	64.35±5.52
<i>Sedum sediforme</i> Pau.	43.60±3.61	12.48±3.79	81.60±4.97
<i>Urtica urens</i> L.	57.09±2.99	77.85±2.42	81.41±0.36
^b LSD _{0.05}	6.44	9.73	6.03

DR = Deoxyribose; ASC = Ascorbate.

^a Percentage of inhibition due to the extracts at the final concentration of 5 mg/ml.

^b LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

They have a similar inhibition percentage such as other spices like anise, ginger, licorice, mint, and nutmeg (Murcia et al., 2004) and different edible wild fruits (*Rubus ulmifolius* and *Sorbus domestica*) (Egea et al., 2010). From this group, *S. minor* and *U. urens* are the species presenting a higher protector effect of deoxyribose (DR) against oxidative attack, with inhibition percentages of 57.75±9.3% and 57.09±2.99%, respectively. The second group of plants, with *C. albidus*, *O. pes-caprae*, and *Q. ballota*, showed inhibition percentages of oxidative attack lower than 35%, similar to cinnamon and vanilla (Murcia et al., 2004), and thus, they could be considered with a low hydroxyl radical (OH[•]) scavenging potential. Their inhibition percentages are near the synthetic antioxidant BHT and propyl gallate (Egea et al., 2010); therefore, they could be considered a prooxidant in the deoxyribose assay and probably can have a synergistic effect with ASC, increasing the concentration of hydroxyl radicals in the medium and consequently stimulating sugar degradation (Murcia, Jimenez and Martinez-Tome, 2001).

There is increasing evidence that H₂O₂, either directly or indirectly via its reduction product OH[•], may act as a messenger molecule in the synthesis and activation of several inflammatory mediators. The ability of different extracts of edible plants to scavenge H₂O₂

is shown in Table 2. *M. nodiflorum*, *S. sediforme*, and *U. urens* exhibited the highest activity, similar to some wild edible fruits like *Rosa canina* and *Crataegus monogyna* (Egea et al., 2010). However, *M. sylvestris* (immature fruits) and *O. pes-caprae* had a very low capacity for scavenging H_2O_2 (10.05 and 4.09% of inhibition, respectively), similar, in this case, to other wild edible fruits like *Crataegus azarolus* and *S. domestica* (Egea et al., 2010). In this assay, N-acetyl-L-cysteine, with a 97.04% hydrogen peroxide scavenging ability, was used as positive hydrogen peroxide scavenger control.

Considering the activity obtained in the different antioxidant assays, no relationship was found between TAA and the ability to capture the $OH^\bullet$ and H_2O_2 radicals. The differences shown by the same plants in different antioxidant tests may be due to the different reaction media and the different chemical natures of the radical species generated (Murcia et al., 2004). The different antioxidant activity of the fourteen extracts from the wild edible plants may be due to their different antioxidant composition.

Organic acids

Different organic acids were identified and quantified: oxalic, citric, malic, and shikimic (**Figure 2**). Oxalic acid is abundant in many leafy vegetables and, in general, a very wide variability in this organic acid content has been found in the literature (Guil et al., 1997; Sánchez-Mata et al., 2012). In this study, very high levels of oxalic acid were found in *O. pes-caprae* (1148 ± 67.9 mg 100 g^{-1} FW) and in both *Mesembryanthemum* species, around 900 mg 100 g^{-1} FW. These levels are significantly greater than those found in the other species of this study ($p < 0.05$). The amount of oxalic acid is higher than that of *Beta maritima* (around 500 mg 100 g^{-1} FW), considered by Guil et al. (1997) and Sánchez-Mata et al. (2012) as a species with a high oxalic acid content. In the present study, low oxalic acid levels were found in other species, like *S. minor*, with 103.28 ± 5.18 mg 100 g^{-1} FW, and the species presenting less than 30 mg 100 g^{-1} FW were *F. vulgare*, *U. urens*, *A. acutifolius*, and *C. maritimum*. Sanchez-Mata et al. (2012) reported oxalic acid levels of 123.82 and 402.83 mg 100 g^{-1} FW in wild *F. vulgare* from different origins, and Souci et al. (2008) indicated a level of 5 mg 100 g^{-1} FW in cultivated *F. vulgare*. Oxalic acid has quite a low toxicity, with 5 g as the minimal lethal dose for an adult. However, the presence of oxalates in plants can interfere with the absorption of Ca and contribute to the formation of oxalate kidney calculi (Guil et al. 1997). For this reason, ingesting high levels of this organic acid is not desirable, although *O. pes-caprae* and both *Mesembryanthemum* species could be consumed in small amounts in salads.

Figure 2: Oxalic acid (Ox), citric acid (Ct), malic acid (MI), and shikimic acid (Sk) (mg 100 g⁻¹ FW) of different wild edible plants. Data are the mean $\pm$ SE. Inner figure: HPLC - profile of oxalic acid in *M. cristalinum*. Data are the mean $\pm$ SE. LSD_{0.05} Ox=26.43; Ct=56.36; MI=63.65; Sk=2.12. LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

In most plant species, the main acids are citric and malic (Oliveira et al., 2008; Sanchez-Mata et al., 2012). In the present work and of all the species studied, *S. sediforme* presented the highest concentration of citric and malic acids, 1717 $\pm$ 8.97 and 1508 $\pm$ 161 mg 100 g⁻¹ FW, respectively ($p < 0.05$). Other species with remarkable amounts of citric acid were *M. cristalinum*, *M. sylvestris* (leaves), and *U. urens* with 408 $\pm$ 15, 561.91 $\pm$ 26 and 458.1 $\pm$ 9 mg 100 g⁻¹ FW, respectively. *A. acutifolius*, with 362.5 $\pm$ 25.7 mg 100g⁻¹ FW, had similar levels of citric acid to those found by Souci et al. (2008) in *Asparagus officinalis* (350 mg 100 g⁻¹). An indirect contribution of citric acid to the entire antiradical activity of some berries could be related to its inhibition properties towards phenolase and polyphenol oxidase enzymes, which are very important for regulating the concentration of polyphenols (Jiang et al., 2004). Therefore, it is possible that citric and malic acids indirectly contribute to the high antioxidant capacity of some of the considered species like *S. sediforme* (Figure 1). In relation to shikimic acid, the quantities found in *S. sediforme* and *C. albidus* were 135.3 $\pm$ 3.3 and 100.3 $\pm$ 4.8 mg 100 g⁻¹ FW. This organic acid is important as most of the phenolic compounds derive from the shikimic acid pathway.

Mineral composition

Mineral elements were found to vary widely depending on the species. Results from the mineral analysis are given in mg per 100 g of dry weight (Table 3). As expected, the Na content was found to be greater in halophytic vegetables, ranging from 50 mg 100 g⁻¹ DW in *Allium ampeloprasum* to 12513 and 13231 mg 100 g⁻¹ DW in *M. cristalinum* and *M. nodiflorum*, respectively. These last values indicate that those species should be consumed without salt addition. The potassium concentration was high in most cases. The lowest value, 980 mg 100 g⁻¹ DW, was found in *Q. ballota*, and there were values above 4000 mg 100 g⁻¹ DW in *A. acutifolius*, *F. vulgare*, leaves of *M. sylvestris*, *M. cristalinum*, and *O. pes-caprae*. That K concentration resulted much higher than in banana (1500 mg K 100 g⁻¹ DW, USDA-ARS, 1998), a fruit considered to have a high K content.

Calcium and Mg nutrition in humans are important concerns due to the critical role they play in bone development and structure, and in cellular metabolism. Dairy products supply a majority of human dietary Ca and Mg requirements; however, certain vegetables can also provide significant amounts of these minerals (Farnham et al., 2000). Spinach has a high Ca concentration, but this occurs in association with oxalates that bind Ca and decrease the mineral's bioavailability. Other vegetables, like broccoli, have relatively high Ca concentrations and low levels of the constituent that renders Ca unavailable. Broccoli has been cited in the popular press as one of the best vegetable sources of Ca in the human diet. Farnham et al. (2000) indicated an average value of 340 mg 100 g⁻¹ DW of Ca in different varieties of broccoli. Considering the species in the present work, the lowest Ca content was found in *Allium* and *Asparagus* (373 and 367 mg 100 g⁻¹ DW, respectively); medium values ranged from 2858 to 3355 mg 100 g⁻¹ DW in *M. cristalinum*, *M. nodiflorum*, *C. maritimum*, and *F. vulgare*, and the highest content was found in *S. sediforme* and *U. urens* (8112 and 9275 mg 100 g⁻¹ DW, respectively). Therefore, these species present a much higher Ca concentration than broccoli, and the consumption of 100 g FW of the last two species would satisfy daily Ca requirements (800 mg). The impact of inadequate Mg is not as significant or as well recognized as it is for Ca, and it is considered that no Mg deficiencies are found in normal diets. Magnesium contents varied from 78 mg 100 g⁻¹ DW (*Q. ballota*) to 965 mg 100 g⁻¹ PS in *U. urens*.

The lowest iron concentration was found in *Q. ballota* (1.48 mg) and in *A. ampeloprasum* (2.2 mg), while the highest occurred in *S. minor* (21.2 mg) and *M. sylvestris* leaves (22.48 mg). The Fe content in raw spinach is 2.71 mg 100 g⁻¹ FW (USDA-ARS, 1998), thus around 27 mg 100 g⁻¹ DW, which indicates that *S. minor* and *M. sylvestris* leaves could be good sources of Fe, like spinach. An intake of around 15 mg per day of Zn, a trace element that is especially important for the normal function of the immune system, is required. Zinc was relatively abundant in *Asparagus* (13.82 mg 100 g⁻¹) and in leaves of *M. sylvestris* (13.5 mg 100 g⁻¹), while its level in *Q. ballota* (3.01 mg 100 g⁻¹) was lower than in the remaining the species.

Table 3 Mineral content of different parts of different wild edible plants (mg 100 g⁻¹ DW) (mean ± SE)

Species	Na	K	Ca	Mg	Fe	Zn
<i>Allium ampeloprasum</i> L.	50.03 ± 5.1	2767±291	373.3±34.8	93.33±6.01	2.22±0.05	3.48±0.05
<i>Asparagus acutifolius</i> L.	112.8±5.1	4167±120	366.7±11.7	156.7±7.3	6.08±0.2	13.82±0.67
<i>Cistus albidus</i> L.	103.6±12.6	1200±0	650±43.7	133.3±6.0	7.15±0.14	5.02±0.34
<i>Crithmum maritimum</i> L.	4820±135	1500±115	3137±20.5	435±27.8	8.29±0.85	6.59±0.84
<i>Foeniculum vulgare</i> Mill.	425.6±35.9	4567±20.3	3355±108	318.3±11.7	8.09±0.68	8.35±1.6
<i>Malva sylvestris</i> L. (leaves)	538.5±17.8	4067±120	890±0	110±0	22.48±2.64	13.5±0.62
<i>Malva sylvestris</i> L. (immature fruits)	251±5.1	2033±33	1280±28	333.3±1.7	8.81±2.32	8.06±0.41
<i>M. nodiflorum</i> L.	13231±514	3100±250	2995±53	675±31.2	7.45±3.29	3.24±0.95
<i>M. cristalinum</i> L.	12513±420	4700±200	2858±107	601.7±26.2	13.49±1.84	9.01±0.41
<i>Oxalis pes-caprae</i> L.	297±5.1	4033±240	781.7±34.2	145±8.7	8.47±3.28	3.67±0.49
<i>Quercus ballota</i> Desf.	32.3±1.4	980±22	80.83±8.8	78.3±3.5	1.48±0.1	3.01±0.08
<i>Sanguisorba minor</i> Scop.	67.2±2.5	1850±95	1772±70	586±52	21.2±4.02	4.81±0.17
<i>Sedum sediforme</i> Pau	90.8±16.2	1467±186	8112±641	260±28.9	7.12±0.7	8.20±0.7
<i>Urtica urens</i> L.	169.2±8.9	3000±58	9275±155	965±7.6	9.38±0.7	7.99±1.13
^a LSD _{0.05}	247.49	211.17	250.22	26.81	2.72	1.01

^a LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Sensory properties

From the consumer's point of view, the following species presented a medium sweetness value (between 6 and 8): *A. ampeloprasum*, *A. acutifolius*, *C. albidus*, and *F. vulgare*, while fruits from *Q. ballota* could be considered very sweet (8.5 ± 0.37). These species could be used as ingredients providing sweetness in salads or other meals. On the other hand, in relation to acidity, *C. maritimum* and *O. pes-caprae* showed the maximum levels, with 7 ± 0.58 and 8.75 ± 0.14 , respectively. Most species from this study were not especially bitter.

The most aromatic plants were *A. ampeloprasum*, *A. acutifolius*, *C. maritimum*, *F. vulgare*, and *Q. ballota*, with values higher than 8. *A. ampeloprasum*, *A. acutifolius*, *C. maritimum*, *M. nodiflorum*, *M. cristalinum*, *S. sediforme*, and *U. urens* were considered especially juicy, with values between 6.5 and 8. Moreover, the panel of tasters highlighted some characteristics from the plants that make them suitable for use as supplements to improve taste, aroma, or texture. Three species stood out for their salty taste: *C. maritimum*, *M. nodiflorum*, and *M. cristalinum*, while *A. ampeloprasum* could add a slightly spicy taste to meals, and *O. pes-caprae* and *Q. ballota* could contribute with very acidic or sweet tastes, respectively.

Petals from *C. albidus* were highlighted for their pink colour and *F. vulgare* for its aroma, while the rest of the species (i.e., *S. sediforme*, *M. sylvestris*, *U. urens*) were appreciated for their appearance, different from the remaining commercial vegetables. These plants, like *S. minor*, could be used in salads. However, when the global appreciation was evaluated, only four species obtained a positive value (between 6 and 10): *A. ampeloprasum*, *A. acutifolius*, *M. cristalinum*, and *Q. ballota*. But it is noteworthy that these values (Table 4) are the results from the evaluation of each individual parameter using raw or boiled material. These plants used with condiments, or dressed in salads, may be appreciated more by the consumer.

Table 4 Global assessment by the consumer and most relevant properties for modern diets of different wild edible plants (mean $\pm$ SE).

Species	Mode of consumption by the tester panel ^a	Sweetness ^b	Acidity ^b	Bitterness ^b	Aroma ^b	Juiciness ^b	Global acceptance ^b	Most relevant properties for modern diets ^c
<i>Allium ampeloprasum</i> L.	Boiled	6.88 $\pm$ 0.75	3.13 $\pm$ 0.13	2.75 $\pm$ 0.69	8.63 $\pm$ 0.53	6.88 $\pm$ 0.42	8.13 $\pm$ 0.33	Aroma, spicy taste
<i>Asparagus acutifolius</i> L.	Boiled	7.67 $\pm$ 0.17	2.00 $\pm$ 0.58	5.67 $\pm$ 0.17	8.00 $\pm$ 0.29	7.67 $\pm$ 0.44	9.67 $\pm$ 0.17	Aroma, texture
<i>Cistus albidus</i> L.	Raw	6.40 $\pm$ 0.52	1.20 $\pm$ 0.38	2.00 $\pm$ 0.58	2.40 $\pm$ 0.33	4.60 $\pm$ 0.70	5.80 $\pm$ 0.38	Colour, appearance
<i>Crithmum maritimum</i> L.	Boiled	4.33 $\pm$ 1.01	7.00 $\pm$ 0.58	5.33 $\pm$ 1.36	9.00 $\pm$ 0.29	7.67 $\pm$ 0.73	2.01 $\pm$ 0.50	Texture, salty taste
<i>Foeniculum vulgare</i> Mill.	Boiled	6.38 $\pm$ 0.84	3.13 $\pm$ 0.57	3.25 $\pm$ 1.02	9.00 $\pm$ 0.46	4.00 $\pm$ 0.87	5.13 $\pm$ 0.78	Aroma
<i>Malva sylvestris</i> L. (leaves)	Boiled	5.20 $\pm$ 0.52	1.80 $\pm$ 0.47	2.40 $\pm$ 0.72	4.00 $\pm$ 0.54	5.80 $\pm$ 0.63	5.01 $\pm$ 0.46	Texture, appearance
<i>Malva sylvestris</i> L. (immature fruits)	Raw	5.20 $\pm$ 0.59	4.20 $\pm$ 0.99	4.40 $\pm$ 0.95	3.20 $\pm$ 0.80	5.20 $\pm$ 1.03	5.20 $\pm$ 0.75	Texture
<i>M. nodiflorum</i> L.	Raw	2.25 $\pm$ 0.72	2.00 $\pm$ 0.78	1.50 $\pm$ 0.29	4.25 $\pm$ 0.60	6.50 $\pm$ 0.76	5.02 $\pm$ 0.24	Texture, salty taste
<i>M. cristalinum</i> L.	Raw	2.00 $\pm$ 0.58	3.00 $\pm$ 0.85	1.25 $\pm$ 0.55	5.25 $\pm$ 0.49	8.00 $\pm$ 0.24	6.00 $\pm$ 0.24	Texture, salty taste
<i>Oxalis pes-caprae</i> L.	Raw	1.75 $\pm$ 0.28	8.75 $\pm$ 0.14	4.00 $\pm$ 1.08	3.75 $\pm$ 0.49	5.50 $\pm$ 0.76	4.00 $\pm$ 0.62	Texture, acid taste
<i>Quercus ballota</i> Desf.	Boiled	8.5 $\pm$ 0.37	0.75 $\pm$ 0.14	2.75 $\pm$ 0.28	9.50 $\pm$ 0.17	2.75 $\pm$ 0.28	9.25 $\pm$ 0.28	Aroma, sweet taste
<i>Sanguisorba minor</i> Scop.	Raw	2.60 $\pm$ 0.44	3.00 $\pm$ 0.54	4.40 $\pm$ 1.01	2.60 $\pm$ 0.44	3.40 $\pm$ 0.39	4.8 $\pm$ 0.12	Appearance
<i>Sedum sediforme</i> Pau	Boiled	2.00 $\pm$ 0.29	4.67 $\pm$ 0.17	5.33 $\pm$ 0.17	7.33 $\pm$ 0.17	7.33 $\pm$ 0.17	1.67 $\pm$ 0.17	Texture, appearance
<i>Urtica urens</i> L.	Boiled	5.25 $\pm$ 0.86	4.00 $\pm$ 0.75	3.75 $\pm$ 0.76	6.25 $\pm$ 0.60	6.50 $\pm$ 0.69	5.00 $\pm$ 0.47	Texture
^d LSD _{0.05}		0.84	0.78	1.06	0.65	0.86	0.61	

^a Mode of consumption by the tester panel (raw or boiled).

^b Sweetness, acidity, bitterness, aroma, juiciness, global acceptance (from 0, minimum value of sweetness to 10, the maximum value).

^c Most relevant properties for creative cuisine were determined by the tester panel.

CONCLUSIONS

In summary, taking into account both total antioxidant activity and the phenolic content of the plants analysed, *S. minor*, *Q. ballota*, *S. sediforme*, and *C. albidus* could be used as nutritional supplements in functional foods with high antioxidant activity and as natural antioxidants to replace synthetic ones. In addition, *S. sediforme* also provides a high amount of calcium to the diet, while *S. minor* and the pink flowers of *C. albidus* would provide colour and enhance the appearance of salads. Both *Mesembryanthemum* species and *O. pes-caprae* would only be recommended in small quantities due to their high oxalic acid content, although they would provide pleasant salty and acidic tastes, respectively. Moreover, these wild plants have a greater mineral content than some cultivated vegetables. Thus, the consumption of the investigated plant species could provide health benefits due to their high antioxidant and nutritional values as well as their sensorial properties. These plants could be used to improve ingredient diversity in modern cuisine.

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REFERENCES

- Cho YS, Yeum KJ, Chen CY, Beretta G, Tang G, Krinsky N, Yoon S, Lee-Kim YC, Blumberg JB, Russell RM. (2007). Phytonutrients affecting hydrophilic and lipophilic antioxidant activities in fruits, vegetables and legumes. *J Sci Food and Agriculture* 87:1096-107.
- Dall'Acqua S, Cervellati R, Loi MC, Innocenti G. (2008). Evaluation of in vitro antioxidant properties of some traditional Sardinian medicinal plants: investigation of the high antioxidant capacity of *Rubus ulmifolius*. *Food Chemistry* 106:745-749.
- Doumett S, Fibbi D, Cincinelli A, Giordani E, Nin S, Del Bubb, M. (2011). Comparison of nutritional and nutraceutical properties in cultivated fruits of *Fragaria vesca* L. produced in Italy. *Food Research International* 44:1209-1216.
- Egea I, Sánchez-Bel P, Romojaro F, Pretel MT. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods for Human Nutrition* 65:121-129.
- Farnham MW, Grusak MA, Wang M. (2000). Calcium and Magnesium Concentration of Inbred and Hybrid Broccoli Heads. *Journal of the American Society for Horticultural Science* 125(3): 344-349.
- Fernández-Panchón MS, Villano D, Troncoso AM, García-Parrilla MC. (2008). Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. *Critical Reviews in Food Science and Nutrition* 48:649-67.
- García-Alonso M, De Pascual TS, Santos-Buelga C, Rivas-Gonzalo JC. (2004). Evaluation of the antioxidant properties of fruit. *Food Chemistry* 84:13-18.

- Guil JL, Rodríguez I, Torija ME. (1997). Nutritional and toxic factors in selected wild edible plants. *Plant Foods for Human Nutrition* 51(2):99-107.
- Hedrick UP. (1972). *Sturtevant's Edible Plants of the World*. Dover Publications. New York.
- Jiang Y, Penb L, Lia J. (2004). Use of citric acid for shelf life and quality maintenance of fresh-cut Chinese water chestnut. *Journal of Food Engineering* 63(3):325-328.
- Leonti M. (2012). The co-evolutionary perspective of the food-medicine continuum and wild gathered and cultivated vegetables. *Genetic Resources and Crop Evolution* 59:1295-1302.
- Łuczaj Ł, Pieroni A, Tardío J, Pardo-de-Santayana M, Sõukand R, Svanberg I, Kalle R. (2012). Wild food plant use in 21st century Europe: the disappearance of old traditions and the search for new cuisines involving wild edibles. *Acta Societatis Botanicorum Poloniae* 81(4): 359-370.
- Marinova D, Ribarova F, Atanassova M. (2005). Total phenolics and total flavonoids in Bulgaria fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy* 40: 255-260.
- Martins D, Barros L, Carvalho AM, Ferreira IC. (2011). Nutritional and in vitro antioxidant properties of edible wild greens in Iberian Peninsula traditional diet. *Food Chemistry* 125(2):488-494.
- Mlcek J, Rop O. (2011). Fresh edible flowers of ornamental plants - A new source of nutraceutical foods. *Trends in Food Science and Technology* 22:561-569.
- Murcia MA, Jimenez AM, Martinez-Tome M. (2001). Evaluation of the Antioxidant Properties of Mediterranean and Tropical Fruits Compared with Common Food Additives. *Journal of Food Protect* 64:2037-2046.
- Murcia MA, Egea I, Romojaro F, Parras P, Jimenez AM, Martínez-Tome M. (2004). Antioxidant Evaluation in Dessert Spices Compared with Common Food Additives. Influence of Irradiation Procedure. *Journal Agricultural Food Chemistry* 5:1872-1881.
- Oliveira AP, Pereira JA, Andrade PB, Valentão P, Seabra RM, Silva BM. (2008). Organic acids composition of *Cydonia oblonga* Miller leaf. *Food Chemistry* 111:393-399.
- Proteggente AR, Pannala AS, Paganga G, Van Buren L, Wagner E, Wiseman S. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C Composition. *Free Radical Research* 36(2):217-233.
- Rivera D, Obón C, Heinrich M, Inocencio C, Verde A, Fajardo J. (2006). Gathered Mediterranean Food Plants - Ethnobotanical investigations and historical development. In Heinrich M, Müller WE and Galli C, eds. *Local Mediterranean Food Plants and Nutraceuticals*. Basel, Karger: Forum Nutrition 18-74.
- Salvatore S, Pellegrini N, Brenna OV, Del Rio D, Frasca G, Brighenti F, Tumino R. (2005). Antioxidant characterization of some Sicilian edible wild greens. *Journal of Agricultural and Food Chemistry* 53(24):9465-9471.
- Sánchez-Mata MC, Cabrera-Loera RD, Morales P, Fernández-Ruiz V, Cámara M, Díez-Marqués C, Pardo-de-Santayana M, Tardío J. (2012). Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds. *Genetic Resources and Crop Evolution* 59(3):431-443.
- Serrano M, Díaz-Mula HM, Zapata PJ, Castillo S, Guillén F, Martínez-Romero D, Valverde JM, Valero D. (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.
- Serrano M, Guillén F, Martínez-Romero D, Castillo S, Valero D. (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agricultural and Food Chemistry* 53:2741-2745.
- Singleton VL, Orthofer R, Lamuela-Raventos RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods in Enzymology* 299:152-178.
- Souci SW, Fachmann W, Kraut H. (2008). Food composition and nutrition tables. (7th revised and completed edition). Stuttgart, Medpharm Scientific Publishers.
- Surveswaran S, Cai YZ, Corke H, Sun M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry* 102:938-953.
- Tardío J, Pardo de Santayana M, Morales R. (2006). Ethnobotanical review of wild edible plants in Spain. *Botanical Journal of the Linnean Society* 152:27-71.
- The Plant List (2012) <http://www.theplantlist.org/> (Accessed 10 July 2012).
- U.S. Department of Agriculture Agricultural research Service. (1998). USDA nutrient database for standard reference, release 12. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>
- Vanzani P, Rossetto M, De Marco V, Sacchetti LE, Paoletti MG, Rigo A. (2011). Wild Mediterranean plants as traditional food: a valuable source of antioxidants. *Journal of Food Science* 76(1): 46-51.

Chapter 3

Wild edible plants as potential antioxidant or nutritional supplements for beverages minimally processed

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Abstract

Wild edible plants (WEP) with high antioxidant capacity, *Rosa canina* fruits, *Quercus ballota* acorns and *Sanguisorba minor* (young leaves and stems), were added to orange and kiwifruit juices for increase the nutritional properties. The addition of WEP to orange juice (OJ) and kiwifruit cremogenate (KC) does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued. The beverages minimally processed with higher TSS were the matrices fortified with *Q. ballota*, the same that were considered sweetest by the judges. The addition of WEP to OJ did not significantly change the levels of vitamin C (ascorbic acid plus dehydroascorbic acid). The addition of *R. canina* and *S. minor* increased the content of phenolic compounds and antioxidant capacity in both matrices (OJ and KC) in more than 30 %. Beverages with a high interest for consumers and with high antioxidant properties have been obtained.

Keywords: wild edible plants, nutritional supplement, antioxidant potential, fruit, minimal processing

INTRODUCTION

During the last years, both food quality and its effects on human health has become a fundamental issue. As a result, many different research works about the functional properties of different foods, plant extracts and natural ingredients have been developed (Serrano et al., 2009). This new awareness have taken food industry to focus the attention on the development of functional or nutraceutical foods with beneficial properties for health (Lavecchia, Rea, Antonacci and Giardi, 2013). Some years ago, the compounds with nutraceutical activity added to foods were usually chemically produced, but new demands for a sustainable life have made that the food industry is now using natural compounds, mainly those derived from plants. Therefore, we can now find in the market a great variety of functional products consisting of food matrices to which extracts of botanical species with known functional and nutraceutical properties have been added (González-Molina, Moreno and García-Viguera, 2008).

In this regard, there is a great variety of wild non-cultivated plants that have been employed in the diet and the traditional medicine of the Mediterranean region (Pardo de Santayana, Tardío and Morales, 2005), but their use has now decreased, an even disappeared, as a consequence of the industrial revolution. Among them, *Rosa canina* L., *Quercus ballota* Desf. and *Sanguisorba minor* Scop., are good candidates for their study as possible functional ingredients due to the nutraceutical and therapeutic properties that many recent studies have found in them. *R. canina* is a significant source of tocopherols, flavonoids, carotenoids and ascorbic acid (Barros, Carvalho, and Ferreira, 2011) and this composition is probably the reason for its high antioxidant capacity (Egea, Sánchez-Bel, Romojaro and Pretel, 2010). *R. canina* also presents anti-inflammatory (Lattanzio et al., 2011) and antibacterial activities (Yilmaz and Ercisli, 2011), anticholinesterase capacity (Boğa, Hacıbekiroğlu, and Kolak, 2011) and antidiarrhoeal properties (Mandade, Choudhury, Harsulkar and Wakade, 2011). On the other side, the fruits of *Q. ballota* are rich in α - and γ -tocopherol and phenolic compounds, especially hydrolysable tannins (Tejerina, García-Torres, Cabeza de Vaca, Vázquez and Cava, 2011). *Q. ballota* also presents a high antioxidant capacity (Tejerina et al., 2011; Romojaro, Botella, Obón and Pretel, 2013), and the antioxidant activity of the acorn powder has been even characterised in order to be used as a new natural antioxidant for fats and oils (Lee, Jeong and Oh, 1992). Regarding *S. minor*, it has been already shown that its young leaves and stems have many and varied phenolic compounds that are derivatives of caffeic acid, apigenin, quercetins, kaempferol with a high antioxidant and antimycotic activities (Gatto et al., 2011; Romojaro et al., 2013).

Fruit juices are usually employed as food matrices for the production of functional foods. In fact, recent studies have shown that the beneficial properties of fruit juices can be increased by adding antioxidant sources (Gonzalez-Molina et al., 2008). Orange (*Citrus cinensis*) is a common raw material for the world fruit juice industry and its juice has a

high nutritional value due to bioactive compounds, as well as an appreciated flavour and aroma (Pala and Toklucu, 2013). The kiwifruit has also a great potential for industrial exploitation, mostly due to its composition rich in phytonutrients (Tavarini, Degl'Innocentia, Remorinib, Massaib and Guidi, 2008). However, the obtaining of a good quality processed kiwifruit is not simple and the possibility to have an industrial valorisation of the fruit is yet away to be found. The main goal of the kiwifruit processing is to obtain safe and stable products able to retain as much as possible the peculiarity of fresh fruit, including green colour, aroma, nutritional value and structural characteristics. One of the main limitations for obtaining kiwifruit minimally processed is the difficulty for maintaining the original green colour which, due to oxidation, quickly acquires brownish tonalities (Cassano and Drioli, 2007; Benlloch-Tinoco, Igual, Rodrigo and Martínez-Navarrete, 2013).

Food industry is currently focused on the development of novel and minimally processed products with improved quality. This type of products has been traditionally preserved by means of conventional thermal technologies. However, it usually requires the use of high temperatures combined with long process times which has been widely associated with a marked deterioration in food quality, especially with the development of cooked off-flavours, colour alteration and the loss of thermosensitive compounds (Benlloch-Tinoco et al., 2013). For this reason, alternatives to conventional processing technologies are being explored. The addition of extracts of wild plants with high antioxidant capacity could, apart from increasing the nutritional value of the juices, help to stabilize and even improve their valued organoleptic properties.

Considering all the previous background, the main aim is to study if the incorporation of *R. canina*, *Q. ballota* and *S. minor* to orange and kiwifruit juices minimally processed could increase the nutritional properties and stabilize or even improve their organoleptic characteristics.

MATERIALS AND METHODS

Wild Edible Plants (WEP)

The non-cultivated and traditionally collected wild edible plants were harvested in the South East (SE) of Spain. Plants were frozen in liquid nitrogen, lyophilized and powdered using a domestic mixer and, finally, stored at 20 °C until further analysis. Wild edible plants used in this study were *Rosa canina* (fruits), *Quercus ballota* (fruits) and *Sanguisorba minor* (young leaves and stems).

Fruit matrices

For the making of kiwifruit cremogenate (KC), kiwifruit of variety Hayward (*Actinidia deliciosa*) from Galicia (España) was employed. For the making of orange juice

(OJ), oranges of variety Navel Lane-Late (*Citrus sinensis*) from Valencia (España) were employed. Both kiwi and orange fruits were purchased at commercial maturity state. The orange juice was obtained using a domestic squeezer (Princesa, Barcelona, Spain) and the kiwifruit cremogenate was obtained using a domestic blender (Osterizer, Madrid, Spain). Both fruits (kiwi and orange) were processed to make juice at eating ripeness.

Preparation of enriched beverages minimally processed

The lyophilised WEP were added to the fruit matrices: natural orange juice (OJ) and kiwifruit cremogenate (KC) and they were homogenised in polytron (Kinematica AG, Lucerne, Switzerland). Previous experiments performed in our laboratory adding 0.1, 0.5, 1 and 2 g of WEP per 100 mL of matrix, showed that the best organoleptic results were obtained when proportion of 0.5 g 100mL⁻¹ was used. Thus this was the proportion used in the subsequent experiments. Homogenised mixtures were filtered in a gauzed filter and stored at room temperature for 24 hours. Samples were analysed after filtering (time 0) and after 24 h of storage.

Sensory determination

Sensory evaluation was conducted by a selected and trained panel comprising ten judges in accordance with ISO 8586-1:1993. The tasting room was kept at 20 °C and beverages served in tasting glasses certified and coded. The evaluation was done using 10-point structured scales, 10 being the best and 1 the worst quality (colour, acidity, sweetness and aroma). The general acceptance quality attribute was assessed as the measurement of the acceptability of the product by the consumer using a scale from “very unpleasant” (level 1) to “very pleasant” (level 10). The selection and training of tasters, as well as the fitting-out of the tasting room, were carried out according to Sanchez-Bel, Egea, Romojaro and Martinez-Madrid (2008). The purchase intent was also evaluated. Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

Quality parameters (TSS, pH, TA)

The total soluble solids content (TSS) was determined by measuring the refractive index of the juice from the fruit with a digital refractometer (Atago, Tokyo, Japan). TSS content was expressed as percentage. pH was measured in a pHmeter (Crison, Barcelona, Spain). Titratable acidity (TA) was determined by potentiometric titration with 0.1 mol equi L⁻¹ NaOH to pH 8.1. The results were expressed as grams of malic acid per 100 g of fresh weight.

Color quality

Colour was measured with a CR-400 colorimeter (Minolta, Paris, France) and results were expressed in CIELAB units of L*, a* and b*. Hue angle (H) and chroma (C) were computed from L*, a* and b* as $H = \tan^{-1}(b^*/a^*)$ and $C = \sqrt{(a^*)^2 + (b^*)^2}$.

Determination of vitamin C (ascorbic and dehydroascorbic acid)

Fruit ascorbate (ASC) content was determined according to Egea et al. (2010) with some modifications; three millilitres of samples homogenised in 10 mL of 5 g 100 mL⁻¹ cold metaphosphoric acid and stirred continuously for 30 min at 4 °C in darkness, and then centrifuged at 20,000 g for 25 min. The supernatant was passed through a C18 column (Sep-pack plus, Waters, Barcelona, Spain) and a 0.2 µm filter. The filtered supernatant was used for ascorbic acid determination using HPLC (LC-10Atvp, Shimadzu, Barcelona, Spain) with a thermostated ion-exchange column (ION-300) at 30 °C and isocratic elution. The absorbance was recorded with a UV/vis detector at 245 nm. A standard curve in the range 10-100 mg kg⁻¹ ascorbic acid was used. The results were expressed as grams of ascorbic acid per 100 g of fresh weight.

Total ascorbate content was determined after reduction of dehydroascorbate (DHA) to ASC with DTT in a final concentration of 30 mmol L⁻¹ for 8 hours in the dark, and then measured as ASC following re-chromatography. Concentration of DHA was estimated from the difference between total ascorbate pool (ASC plus DHA) and ASC.

Determination of total phenolic compounds

Total phenolic compounds were determined according to Serrano et al. (2009) using (3 g) homogenized in 10 mL of 100 mmol L⁻¹ sodium phosphate buffer containing 150 mmol L⁻¹ NaCl, pH 7.4 on ice. The concentration of the total phenolic compounds in fruit extracts was determined by comparison with the absorbance of analytical grade phenol standard, gallic acid, at different concentrations. The results were expressed as grams of galic acid per 100 g of fresh weight (FW).

Determination of antiradical activity: Trolox equivalent antioxidant capacity (TEAC assay)

The ABTS[•] radical anion solution was generated by incubating, at 60 °C for 6 min, a mixture of 2.5 mmol L⁻¹ 2,2'-azobis(2-amidinopropane) hydrochloride (ABAP) and 20 mmol L⁻¹ 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS²) stock solution in 100 mL of phosphate buffer (100 mmol L⁻¹ phosphate and 150 mmol L⁻¹ NaCl, pH 7.4). Samples (3 g) were homogenized in 10 mL of 100 mmol L⁻¹ sodium phosphate buffer containing 150 mmol L⁻¹ NaCl, pH 7.4 on ice. The supernatant was collected as final extract. 40 µL of each extract were mixed with 1960 µL of the radical solution to measure the antioxidant activity at 734 nm after 6 min of incubation at 36°C. A calibration curve was prepared with different concentrations of Trolox. The TEAC activity was calculated according to Egea et al. (2010) and represents the concentration of Trolox, in µmol L⁻¹, that has the same antioxidant capacity as the analyzed sample (Trolox equivalent).

Statistical analysis

Tests for significant differences were carried out using the general linear model of the SPSS (version 14.0, SPSS Inc., Chicago, USA) statistical package. Analysis of variance (ANOVA) was conducted using the treatments as a statistical parameter, the confidence level being 95 %. Correlations between different parameters were computed as Pearson's correlation coefficient (r). When differences were significant, multiple comparisons test was made using least significant difference test (LSD). Values represent the means $\pm$ SD of three replications for each of three sub-samples per sample.

RESULTS AND DISCUSSION

Consumers' acceptance

The sensory assessment is the main aspect for designing new foods because changes in aroma, texture or colour of the original matrices can significantly affect the consumer's acceptance. Although the overall impression is a function of many attributes and their interactions, it is interesting to identify the particular attributes that move acceptance. The complexity of food is such that it may well be impossible to truly discern the relative contributions of the different sensory inputs to food acceptance (Janzantti, Machado and Monteiro, 2011). In this work, the attribute colour was assessed in different ways according to the matrix (OJ or KC) and the added WEP (*Rosa canina* -*Rc*-, *Quercus ballota* -*Qb*- or *Sanguisorba minor* -*Sm*-) (Table 1). The colour of the OJ fortified with *Rc* was significantly ($p<0.05$) better valued than the control one (119.72 ± 5.31), while the addition of *Qb* did not present differences regarding control OJ (95.87 ± 3.73). The addition of *Sm* to OJ reduced the redness of the orange juice and this fact was negatively assessed by the consumer (39.14 ± 10.59). Regarding KC, consumers positively assessed the increase of green colour intensity provided by *Sm* (124.01 ± 8.73). On the contrary, the addition of *Rc* gave KC a brownish aspect that was significantly worse valued (61.86 ± 9.42) than that of control KC. All matrices were considered less acid than control, regardless of the added WEP. The WEP addition increased the sweetness between 35 and 85 % regarding the unfortified matrices. The addition of WEP to OJ and KC decreased the aroma assessment in most mixtures, although not significantly. In our work, and in keeping with other authors (Tepper, 1993) regarding overall impression, colour was the most significant value because the worst assessed mixtures were OJ with *Sm* and KC with *Rc*, a fact coincident to the worst assessment of colour. The best valued were OJ with *Rc* and KC with *Sm*. Consumers showed a better purchase intent for OJ with *Rc* and KC with *Sm* and *Qb*.

It is worth mentioning the high purchase intent of KC with *Qb* because it would be expected that this enriched beverage show an increase of an astringent and bitter flavour due to the high content of tannins. However, matrices with *Qb* obtained a better overall impression and purchase intent than KC. Some authors have stated that the astringent

flavour can be modified by the addition of other compounds, and that the sweetness can reduce the astringent sensation. In this study, matrices with *Qb* were those obtaining the highest punctuation in sweetness, a fact stating that, despite the high content in tannins of *Qb*, the extra supply of sweetness can avoid the astringent and bitter flavour of the tannins. In general, it could be concluded that the addition of WEP to OJ and KC does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued.

Table 1: Consumer acceptance of orange juice (OJ) and kiwi liquefied (KC) after addition of wild edible plants (WEP). Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

Matrix with WEP ^a	Colour ^b	Acidity ^b	Sweetness ^b	Aroma ^b	Overall impression ^b	Purchase intent ^b
OJ with <i>Rc</i>	119.72±5.31	87.36±5.81	153.57±17.45	84.76±12.42	103.45±8.35	117.62±9.79
OJ with <i>Qb</i>	95.87±3.73	92.08±7.04	184.72±31.93	83.57±6.08	100.87±9.40	95.24±5.53
OJ with <i>Sm</i>	39.14±10.59	85.92±7.95	155.56±35.44	80.95±11.28	80.00±24.34	89.76±18.6
KC with <i>Rc</i>	61.87±9.42	63.45±10.67	135.75±14.75	95.04±5.72	84.01±8.70	65.43±15
KC with <i>Qb</i>	114.34±6.97	84.64±10.76	157.01±30.06	82.54±10.78	125.1±15.5	115.00±14
KC with <i>Sm</i>	124.01±8.73	75.00±6.96	152.38±35.62	105.79±23.85	129.6±11.8	123.00±17
^c LSD (p<0.05)	11.67	10.67	40.44	17.31	29.09	15.17

^aMatrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina Rc*, *Quercus ballota -Qb* or *Sanguisorba minor -Sm*).

^b Colour, acidity, sweetness, aroma, overall impression and purchase intent. (100 the punctuation obtained by the unfortified matrix).

^c LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Changes in quality parameters

The addition of WEP to matrices (OJ and KC) did not significantly change ($p<0.05$) the pH or the TA of the mixtures (Table 2). The TA of the new beverages ranged between 1.66 ± 0.04 of OJ with *Sm* and 1.34 ± 0.04 of KC with *Sm*. The pH of the mixtures remained between 3.5 and 4.25, within the levels found by other authors (Barros et al., 2011). However, the TSS with WEP was higher in all cases than in control matrices, and they coincide with the sweetness value given by judges. Therefore, while the TSS of the control OJ did not exceed 10.67 °Brix, when the juice was fortified with WEP this parameter was higher, ranging between 11.11 ± 0.15 in freshly made OJ with *Sm* and 11.83 ± 0.11 in OJ with *Qb* after 24 hours of storage at 20 °C. The TSS of KC was also higher in matrices enriched

with WEP than in control KC. While TSS of control KC did not exceed 13.93 °Brix, the TSS of the fortified mixtures of kiwifruit ranged between 14.30±0.15 °Brix of freshly made KC with *Sm* and 14.94±0.24 of KC with *Qb* after 24 hours of storage at 20 °C. In general, the beverages minimally processed with higher TSS were the matrices fortified with *Qb*, the same that were considered sweetest by the judges (Table 2). This increase of TSS is an interesting characteristic because it can increase the sweetness of the beverages without adding sugars and, therefore, avoiding the increase of empty calories (Guenther, Reedy and Krebs-Smith, 2008), a more and more demanded issue by consumers.

Table 2: Acidity, pH and soluble solids of orange juice (OJ) and kiwifruit cremogenate (KC) after addition of wild edible plants (WEP).

Matrix with WEP ^a	Time (h) ^b	TA (g 100g ⁻¹)	pH	TSS (Brix)
OJ Control	0	1.61±0.02	3.61±0.20	10.63±0.11
OJ with <i>Rc</i>		1.54±0.03	3.73±0.03	11.10±0.12
OJ with <i>Qb</i>		1.58±0.05	3.74±0.07	11.43±0.07
OJ with <i>Sm</i>		1.66±0.04	3.45±0.30	11.01±0.15
OJ Control	24	1.65±0.01	3.64±0.14	10.67±0.22
OJ with <i>Rc</i>		1.57±0.03	3.43±0.02	11.30±0.10
OJ with <i>Qb</i>		1.60±0.04	3.39±0.02	10.50±0.06
OJ with <i>Sm</i>		1.63±0.08	3.57±0.06	10.83±0.09
^c LSD (p<0.05)		0.06	0.20	0.18
KC Control	0	1.40±0.01	3.67±0.42	13.53±0.12
KC with <i>Rc</i>		1.41±0.06	4.07±0.13	14.78±0.38
KC with <i>Qb</i>		1.34±0.05	3.61±0.26	14.38±0.23
KC with <i>Sm</i>		1.34±0.04	4.25±0.26	14.33±0.07
KC Control	24	1.39±0.02	3.6±0.23	13.93±0.18
KC with <i>Rc</i>		1.44±0.03	3.74±0.29	14.87±0.30
KC with <i>Qb</i>		1.37±0.04	3.70±0.29	14.43±0.24
KC with <i>Sm</i>		1.35±0.03	3.64±0.25	14.30±0.15
^c LSD (p<0.05)		0.05	0.39	2.23

Values represent the means ± SD of three replications for each of three sub samples per sample.

^a Matrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina Rc*, *Quercus ballota Qb* or *Sanguisorba minor Sm*).

^b Time (0h and after 24h a 20°C).

^c LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Changes in colour quality

The enrichment of OJ and KC with WEP with high antioxidant capacity can change colour, although it can also contribute to its stability against oxidation. In OJ, the addition of *Rc* significantly increased ($p<0.05$) the orange colour of the juice by increasing the redness (CIEa* value) and the yellowness (CIEb* value) (Table 3). On the contrary, the addition of *Qb* did not change the CIEa*, although it increased the lightness (CIEL* value) and decreased the intensity of the orange colour. The addition of *Sm* significantly changed ($p<0.05$) colour (CIEa* more negative than that of control) due to the chlorophyll of leaves and young shoots of this plant that gives the juice a colour less valued by consumers (Table 2). It is worth mentioning that the addition of *Rc* to KC gave a brownish colour to the mixture (CIEa* -4.53 ± 0.04 and Hue angle 105.36 ± 0.09), coincident with a low value given by consumers (61.87 % regarding control). However, the resulting colour of the mixture of *Qb* or *Sm* with KC, with lower CIEa* and CIEb* values, was better valued by consumers than that of control (Table 2). The mixtures of different matrices change the profile of the pigments (Lemmens, Van Buggenhout, Van Loey and Hendrickx, 2010). So WEP with high contents of chlorophyll and brown pigments are more suitable for KC, while those rich in carotenoids (orange and red pigments) are more suitable for OJ in order to obtain a product well valued by consumers.

After 24 hours at 20 °C, the orange colour of OJ was significantly ($p<0.05$) less intense, as it can be shown by the decrease of CIEa* and CIEb* (Table 3) probably caused by the degradation of β -cryptoxanthin, antheraxanthin and violaxanthin, the main responsible carotenoids for the colour of the orange juice (Carmona, Zacarias & Rodrigo, 2012). Although the addition of *Rc* to OJ did not avoid the degradation of carotenoids of the juice, the OJ with *Rc* preserved an intense red colour due to the increase in the mixture of β -carotene found in *Rosa canina* (97.77 mg 100 g⁻¹ DW) (Barros et al., 2011). It is already known that the stability of red colour is a big problem in food processing (Rein and Heinomen, 2004) and, therefore, the addition of *Rc* as colouring agent to certain matrices could compensate for the loss of pigments during the processing and could enrich the juices with other bioactive compounds found in *R. canina* (Egea et al., 2010; Barros et al., 2011). On the other side, the addition of *Qb* to the OJ implied a quicker loss of red colour of the OJ regarding control, while the OJ with *Sm* practically reached the same colour than the control one, it won redness (less negative CIEa* value). Therefore, it seems that *Qb* potentiates the degradation of the characteristic carotenoids of the orange juice and the OJ potentiates the degradation of the chlorophyll of *Sm*.

The colour of KC changed after 24 hours at 20 °C, with a significant decrease of the Hue angle (more than 70° of difference between initial and final values), a fact showing a high percentage of darkening (Gonzalez-Molina et al., 2008). The addition of WEP to KC affects in different ways according to the species. While the addition of *Sm* does not reduce darkening (Hue angle value similar to the control), the addition of *Rc* and *Qb*

significantly reduce darkening of KC and, particularly, the addition of *Rc* reduce darkening to half. Therefore, *Qb* and *Rc* could be used as an alternative to synthetic compounds for minimizing the darkening in other products that are minimally processed like vegetables cremogenate. However, the use of these species as anti-darkening agents would need a deeper study because the colour stabilization depends upon the structure and concentration of the pigment, pH, temperature, presence of co-pigments, ascorbic acid, metallic ions, etc (Wicklund et al., 2005).

Table 3: Colour of orange juice (OJ) and kiwifruit cremogenate (KC) after enrichment with wild edible plants (WEP).

Matrix with WEP ^a	Time (h) ^b	CIEL*	CIEa*	CIEb*	Croma	Hue
OJ Control	0	38.08±0.28	-5.58±0.04	18.47±0.45	19.30±0.44	106.83±0.27
OJ with <i>Rc</i>		39.82±0.19	-2.78±0.01	20.26±0.09	20.45±0.08	97.81±0.02
OJ with <i>Qb</i>		43.41±0.27	-5.38±0.03	22.37±0.31	23.01±0.31	103.53±0.15
OJ with <i>Sm</i>		38.22±0.18	-7.55±0.14	17.24±0.44	18.83±0.45	113.66±0.18
OJ Control	24	38.82±0.12	-5.92±0.12	17.92±0.37	18.87±0.39	108.29±0.21
OJ with <i>Rc</i>		39.58±0.33	-2.96±0.08	19.04±0.18	19.27±0.19	98.82±0.15
OJ with <i>Qb</i>		42.85±0.12	-6.15±0.19	20.80±0.53	21.70±0.53	106.49±0.47
OJ with <i>Sm</i>		38.74±0.78	-5.41±0.24	17.36±0.89	18.18±0.92	107.32±0.18
^c LSD (p<0.05)		0.49	0.18	0.65	0.66	0.33
KC Control	0	43.75±0.70	-7.41±0.08	16.12±0.22	17.74±0.22	114.69±0.32
KC with <i>Rc</i>		42.70±0.03	-4.53±0.04	16.48±0.21	17.09±0.22	105.36±0.09
KC with <i>Qb</i>		47.08±0.12	-7.76±0.05	18.06±0.20	19.60±0.21	113.32±0.11
KC with <i>Sm</i>		42.41±0.50	-9.14±0.17	18.75±0.12	20.86±0.15	115.98±0.38
KC Control	24	38.96±0.27	-2.77±1.23	11.39±0.38	19.25±1.00	44.64±1.82
KC with <i>Rc</i>		39.00±0.36	-2.27±0.27	13.24±0.26	32.34±7.32	71.84±12.81
KC with <i>Qb</i>		43.42±0.41	-4.42±0.10	13.26±0.62	34.29±7.61	62.81±16.06
KC with <i>Sm</i>		39.00±0.36	-2.27±0.27	13.24±0.26	18.26±1.31	48.49±11.03
^c LSD (p<0.05)		0.55	0.65	0.44	5.28	11.55

Values represent the means ± SD of three replications for each of three sub samples per sample.

^a Matrix: Orange juice (OJ), Kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina Rc*, *Quercus ballota Qb* o *Sanguisorba minor Sm*).

^b Time (0h and after 24h at 20°C).

^c LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Changes in Vitamin C and polyphenols contents

The addition of WEP to OJ did not significantly change ($p < 0.05$) the levels of vitamin C (ascorbic acid plus dehydroascorbic acid) regarding the non fortified OJ and KC matrices (**Figure 1a, c**). The initial levels of vitamin C in OJ were $124.75 \pm 3.35 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, similar to those found by other authors (Tarrago-Trani, Philips and Cotty, 2012). Although the addition of WEP increases vitamin C between 10 and 15 %, it was not a significant increase. Vitamin C in KC did not change regarding control when WEP were added; the levels ranged around $170 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, similar to those found by other authors (Celik, Sezai and Turgut, 2007). The stability of vitamin C in matrices (OJ and KC) at 20°C during 24 hours did not significantly change ($p < 0.05$). So, in general, the addition of WEP to OJ and KC did not significantly change the content of vitamin C or increase its stability under tested conditions. For nutritional purposes, vitamin C is the sum of AA and dehydroascorbic acid (DHAA); DHAA absorbed from the intestinal tract undergoes intracellular reduction (via NADPH- and glutathione-dependent reductases), rendering it biologically active (Gonzalez-Molina et al., 2008). It must be mentioned that when the matrix is OJ, the proportion of AA (**Figure 1b**) after 24 hours at 20°C significantly increased ($p < 0.05$). AA is susceptible to oxidation in aqueous solution; therefore, temperature, light, pH, the presence of oxygen, metal catalysts (iron, copper) and enzymes such as ascorbate oxidase affect the rate of AA degradation, at a variable rate in different foods (Johnston, Steinberg and Rucker, 2007). It was verified (**Figure 1b embedded**) that, in aqueous solution and under tested conditions (24 hours at 20°C), the speed of degradation of the ascorbic acid is very high during the first hours, regardless the concentration of AA of the initial solution.

The ascorbic acid in OJ or KC (**Figure 1b, d**) did not significantly degrade ($p < 0.05$) after 24 hours at 20°C . The TA and the pH of the matrices contribute to the AA stability because at low pH AA is fully protonated and relatively stable, with maximal stability at 4-6; at $\text{pH} > 6$, AA is reversibly oxidized to DHAA, and DHAA may be further irreversibly oxidized to 2,3-diketo-l-gluconic acid, followed by degradation to other by-products (Johnston et al., 2007). On the other side, OJ is a rich source of flavanons, including hesperidin and narirutin, which are also main phenolic compounds of OJ (Proteggente et al., 2002). Moreover, kiwifruit is rich in polyphenols compared with other fruits (Park et al., 2006). The addition of *Rc* and *Sm* increased the content of phenolic compounds in both matrices (OJ and KC) in more than 30 % due to the fact that both *Rc* and *Sm* have a high concentration of these compounds (Barros et al., 2011; Egea et al., 2010). However, the addition of *Qb* did not significantly change the levels of polyphenols (**Figure 2 a, b**). The high concentration of tannins found in *Qb* could cause the precipitation of tannin acids or tannin-protein complexes responsible for the astringent flavour of the tannins, a flavour that is disgusting to the consumer (Tejerina et al., 2011). The phenolic compounds remained stable during 24 hours at room temperature in all matrices.

Figure 1: Changes in levels of total ascorbate (Vitamin C) and ascorbic acid content of orange juice (OJ), (figures a and b) and Kiwifruit cremogenate (KC) (figures c and d) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* -Qb- o *Sanguisorba minor* -Sm-) at time 0 or after 24 hours at 20°C. Figure 1b embedded shows the degradation rate of ascorbic acid in aqueous solution under the same tested conditions as OJ and KC (24 hours at 20 °C). LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of samples.

Changes in Trolox Equivalent Antioxidant Capacity (TEAC) and relationship with phytonutrient levels

One of the main quality attributes of juices is their antioxidant capacity and, during the last years, this has been a very solid argument for the professionals of the food industry. **Figure 2 (c and d)** shows the antioxidant capacity (TEAC) of OJ and KC, respectively, after their enrichment with different WEP. The initial antioxidant capacity of KC was higher than that of OJ (359.04 ± 46 and 298.07 ± 10.2 equi Trolox $\mu\text{mol L}^{-1}$, respectively). There is a controversial debate in the literature about the influence of vitamin C on the antioxidant capacity of fruits or vegetables (Guo et al., 2003; Tavarini et al., 2008). It is also known that fruits with high antioxidant capacity generally contain more antioxidants and most of these antioxidants has been showed to be phenolic compounds and, in particular, flavonoids (Guo et al., 2003). According to some authors (Proteggente et al., 2002; Tavarini et al., 2008), the antioxidant capacity of OJ mainly results from its ascorbic acid content, while flavanone glycosides have a limited contribution to its antioxidant capacity. In our work, the addition of Qb did not significantly increase the antioxidant capacity of the matrices (OJ and KC), while the

addition of *Rc* increased in 67 and 39 % the antioxidant capacity of OJ and KC, respectively. The antioxidant capacity of OJ and KC with *Sm* also increased in 60 and 30 %, respectively. Coincident to our results, some studies have shown that the activity in the TEAC assay increases with the number of hydroxyl groups of the antioxidant compound, so the antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts under experimental conditions (Hodisan, Socaciu, Ropan and Neamtu, 1997). This relation between total phenols and the antioxidant activity has been found in many fruits and all their derived products (Egea et al., 2010), a fact suggesting that the phenolic compounds could be responsible for the antioxidant properties of the new beverages fortified with *Rc* and *Sm* because there is a good correlation (**Table 4**) between the content in polyphenols of the new beverages and the antioxidant capacity and, however, there is a very low correlation between the content in vitamin C and the antioxidant capacity of the mixtures.

Figure 2: Changes in levels of total polyphenol content (figures a and b) and Trolox Equivalent Antioxidant Capacity (TEAC) (figures c and d) of orange juice (OJ), (figures a and c) and Kiwifruit cremogenate (KC) (figures b and d) fortified with WEP (*Rosa canina* -Rc-, *Quercus ballota* Qb- o *Sanguisorba minor* Sm-) at time 0 or after 24 hours at 20°C. LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of samples.

However, when the new beverages were stored during 24 hours at 20 °C the antioxidant capacity of the beverages fortified with *Rc* and *Sm* significantly decreased, between 10 and 15 % in OJ and between 15 and 25 % in KC. This decrease could be due to reactions of polymerization, condensation or complexation that can cause the formation

of new compounds with a lower antioxidant capacity (Gonzalez-Molina et al., 2008). Moreover, the presence of other antioxidant compounds from *Rc* and *Sm*, such as proteins, which we have not analyzed, could react in the mixtures decreasing the in vitro antioxidant values (Gonzalez-Molina et al., 2008). The antioxidant properties of food matrices are due to the presence in complex mixtures of compounds of varying polarity, such as vitamin C, vitamin E, carotenoids and polyphenols. Due to the potential synergistic action of all these bioactive compounds present in food (Egea et al., 2010) the total antioxidant capacity of some foods has increasingly become a sales argument for professionals in the food industry.

Table 4 Analisis of correlations between total antioxidant activity (TEAC), total poliphenols content and vitamin C in the prepared mixtures.

Antioxidant Capacity (TEAC)				
	OJ ^{0h}	KL ^{0h}	OJ ^{24h}	KL ^{24h}
Phenolics	0.9620 ^{MS}	0.9669 ^{MS}	0.9500 ^{MS}	0.9280 ^{MS}
Vitamin C	0.3095	0.5220	0.1988	0.2091

^{MS} Marginally significant ($P > 0.05$).

CONCLUSIONS

The beverages that result from the addition of wild edible plants with high antioxidant capacity to orange juice or kiwifruit cremogenate are well accepted by consumers, even improving the acceptance of the fresh product, and they improve the content in phenolic compounds and the antioxidant capacity without changing the physico-chemical properties of the matrices. This better acceptance of enriched beverages minimally processed by the consumer was due, to a great extent, to the intensification of the natural colour (in the case of addition of *Rc* to OJ and *Sm* to KC) and to the extra sweetness provided mainly by *Qb* and, to a lesser extent, by *Rc* because the addition of wild edible plants to the matrices increased their content in soluble solids. Beverages minimally processed with a high interest for consumers and with high antioxidant properties have been obtained.

REFERENCES

- Barros, L., Carvalho, A.M. and Ferreira, I. (2011). Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. *Food Research International*, 44 (7), 2233-2236.
- Benlloch-Tinoco, M., Igual, M., Rodrigo, D. and Martínez-Navarrete, N. (2013). Comparison of microwaves and conventional thermal treatment on enzymes activity and antioxidant capacity of kiwifruit puree. *Innovative Food Science and Emerging Technologies*, 19, 166-172.

- Boğa M., Hacıbekiroğlu, I. and Kolak, U. (2011). Antioxidant and anticholinesterase activities of eleven edible plants. *Pharm Biol*, 49(3), 290-295.
- Carmona, L., Zacarias, L. and Rodrigo, M.J. (2012). Stimulation of coloration and carotenoid biosynthesis during postharvest storage of 'Navelina' orange fruit at 12 °C. *Postharvest Biology and Technology*, 74, 108-117.
- Cassano, A. and Drioli, E. (2007). Concentration of clarified kiwifruit juice by osmotic distillation. *Journal of Food Engineering*, 79, 1397-1404.
- Celik, A., Sezai, E. and Turgut, N. (2007). Some physical, pomological and nutritional properties of kiwifruit cv. Hayward. *International Journal of Food Sciences and Nutrition*, 58, 411-418.
- Egea, I., Sánchez-Bel, P., Romojaro, F. and Pretel, M.T. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods For Human Nutrition*, 65, 121-129.
- Gatto, M.A., Ippolito, A., Linsalata, V., Cascarano, N.A., Nigro, F., Vanadia, S. and Di Venere, D. (2011). Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biology and Technology*, 61 (1), 72-82.
- González-Molina, E., Moreno, D.A. and García-Viguera, C., (2008). Aronia-enriched lemon juice: a new highly antioxidant beverage. *J Agric Food Chem*, 56, 11327-11333.
- Guenther, P.M., Reedy, J. and Krebs-Smith, S.M. (2008). Development of the healthy eating index. *J Am Diet Assoc*, 108(11), 1896-901.
- Guo, C.J., Yang, J.J., Wei, J.Y., Li, Y.F., Xu, J., Jiang, Y.G., 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, 23, 1719-1726.
- Hodisan, T., Socaciu, C., Ropan, I. and Neamtu, G. (1997). Carotenoid composition of *Rosa canina* fruits determined by thin-layer chromatography and high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 16, 521-528.
- Janzantti, N.S., Machado, T.V. and Monteiro, M. (2011). Sensory acceptance of juice from FCOJ processing steps. *Journal of Sensory Studies*, 26 (5), 322-330.
- Johnston, C.S., Steinberg, F.M. and Rucker, R.B. (2007). Ascorbic acid. In J. Zempleni, R.B. Rucker, D.B. McCormick, J.W. Suttie (Eds.), *Handbook of Vitamins* (4th ed., pp. 489-520). CRC Press, Boca Raton, FL, USA.
- Lavecchia, T., Rea, G., Antonacci, A. and Giardi, M.T. (2013). Healthy and adverse effects of plant-derived functional metabolites: The need of revealing their content and bioactivity in a complex food matrix. *Critical Reviews in Food Science and Nutrition*, 53, 198-213.
- Lattanzio, F., Greco, E., Carretta, D., Cervellati R., Govonic, P. and Speronia, E. (2011). In vivo anti-inflammatory effect of *Rosa canina* L. extract. *Journal of Ethnopharmacology*, 137: 880-885.
- Lee, M.H., Jeong, J.H. and Oh, M.J. (1992). Antioxidative activity of gallic acid in acorn extract. *Journal of the Korean Society of Food and Nutrition*, 21(6), 693-700.
- Lemmens L., Van Buggenhout, S., Van Loey, A.M. and Hendrickx, M.E. (2010). Particle size reduction leading to cell wall rupture is more important for the β -carotene bioaccessibility of raw compared to thermally processed carrots. *J. Agric. Food Chem*. 58, 12769-12776.

- Mandade, R.J., Choudhury, A., Harsulkar, A. and Wakade, R. (2011). Role of the *Rosa canina* L. leaf extract as an antidiarrheal drug in rodents. *Indian Journal of Pharmacology*, 43, 316-319.
- Pala, Ç.U. and Toklucu, A.K. (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT - Food Science and Technology*, 50(2), 426-431.
- Pardo de Santayana, M., Tardío, J. and Morales, R. (2005). The gathering and consumption of wild edible plants in the Campoo (Cantabria, Spain). *Int J Food Sci Nutr*, 56(7): 529-542.
- Park, Y.S., Junj, S.T., Kang, S.G., Drzewiecki, J., Namiesnik, J., Haruenkit, R., Barasch, D., Trakhtenberg, S. and Gorinstein, S. (2006). In vitro studies of polyphenols, antioxidants and other dietary indices in kiwifruit (*Actinidia deliciosa*). *Int. J. Food Sci. Nutr*, 57, 107-122.
- Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E. and Wiseman, S. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C Composition. *Free Radical Research*, 36(2), 217-233.
- Rein, M.J. and Heinonen, M. (2004). Stability and enhancement of berry juice color. *J. Agric. Food Chem*, 52(10), 3106-3114.
- Romero, A., Botella, M.A., Obón, C. and Pretel, M.T. (2013). Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet. *Int J Food Sci Nutr*, 64(8): 944-952.
- Sanchez-Bel, P., Egea, I., Romero, F. and Martinez-Madrid, M.C. (2008). Sensorial and chemical quality of electron beam irradiated almonds (*Prunus amygdalus*). *LWT-Food Science and Technology*, 41, 442-449.
- Serrano, M., Díaz-Mula, H.M., Zapata, P.J., Castillo, S., Guillén, F., Martínez-Romero, D., Valverde, J.M. and 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.
- Tarrago-Trani, M.T., Philips, K.M. and Cotty, M. (2012). Matrix-specific method validation for quantitative analysis of vitamin C in diverse foods. *Journal of Food Composition and Analysis*, 26, 12-25.
- Tavarini, S., Degl'Innocentia, E., Remorinib, D., Massaib, R. and Guidi, L. (2008). Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chemistry*, 107, 282-288.
- Tejerina, D., García-Torres, S., Cabeza de Vaca, M., Vázquez, F.M. and Cava, R. (2011). Acorns (*Quercus rotundifolia* Lam.) and grass as natural sources of antioxidants and fatty acids in the "montanera" feeding of Iberian pig: Intra- and inter-annual variations. *Food Chemistry*, 124, 997-1004.
- Tepper, B. J. (1993). Effects of a slight color variation on consumer acceptance of orange juice. *Journal of Sensory Studies*, 8, 145-154.
- Wicklund, T., Rosenfeld, H.J., Martinsen, B.K., Sundfør, M.W., Lea, P., Bruun, T., Blomhoff, R. and Haffner, K. (2005). Antioxidant capacity and colour of strawberry jam as influenced by cultivar and storage conditions. *Food Sci. Technol*, 38, 387-391.
- Yilmaz, S.O. and Ercisli S. (2011). Antibacterial and antioxidant activity of fruits of some rose species from Turkey. *Romanian Biotechnological letters*, 16, 6407-6411.

Chapter 4

Wild edible plants as potential antioxidants in vegetables oils

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Wild edible plants as potential antioxidants in vegetables oils

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Abstract

The effect of the addition of fruits from *Rosa canina* and *Quercus ballota* and leaves of *Sanguisorba minor* to sunflower, seed and olive oils on lipid oxidation during the frying process was analyzed. The three underutilised edible plants increased the oxidative stability of the sunflower oil, since the malondialdehyde (MDA) content was significantly decreased with respect to values of the control sunflower oil after the heating process. However, in olive oil the effect of these edible plants on decreasing the lipid peroxidation was only evident for the highest concentration, while in seed oil the addition of the edible plant showed a pro-oxidant effect. Thus, these wild edible plants could be used to enrich vegetable oils with low content of natural antioxidant, such as sunflower oil and avoid or decrease the use of synthetic antioxidant.

Keywords: wild edible plants, edible vegetable oil, antioxidant potential, *Rosa canina*, *Quercus ballota*, *Sanguisorba minor*, underutilised vegetables

INTRODUCTION

Edible vegetable oils such as sunflower oil, seed oil, and olive oil have an important place in human diet, since their use in culinary processes is a constant. However, during frying, a gradual deterioration of oil quality occurs due to lipid oxidative decomposition reactions leading to lowering quality and nutritional value of foods, with consequences for the health of the consumer, causing aging, membrane damage, and numerous diseases, such as chronic inflammation, neurodegenerative diseases, adult respiratory distress syndrome, atherogenesis, diabetes, and different types of cancer [1]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ), are widely used in the food industry to avoid oxidation [2] due to their low cost and good performance, although their safety, has been questioned due to carcinogenic effect [3]. According to the European Bulletin (Order of 24 August 2007) [4] the maximum dose allowed of these artificial additives in frying oils is 200 mg kg⁻¹ of oil.

Under these circumstances, research on development of safe natural antioxidants, are therefore essential. Recently it has been shown that *Nigella sativa* seed extract could be an interesting alternative to the use of synthetic antioxidants, since the addition of Nigella seed extract to sunflower oil improved their thermal stability and shelf-life [5]. Accordingly, Cordeiro et al. [6] have shown that extract of *Rosmarinus officinalis* added to sunflower, corn and soybean oils displayed a more effective anti-oxidative action than TBHQ and it was stable at the frying temperature of the oils, unlike the majority of the antioxidants commonly used. Moreover, even in olive oils which content high concentration of natural antioxidants, the addition of oregano essential oils lead to a protective effect on primary lipid oxidation [7].

Recent phytochemical studies have revealed that fruits from *Rosa canina* L. contain high amounts of vitamin C, carotenoids and polyphenols making them a suitable source of antioxidants to be used commercially to retard rancidity in fatty materials in food manufacturing [8, 9]. Accordingly, acorns from *Quercus spp* also possess biologically active substances, such as α - and γ -tocopherol and phenolics compounds, specially tannins, with proved antioxidant and free-radical-scavenging capacities [10, 11]. Finally, leaves and shoot from *Sanguisorba minor* Scop. (Sm) have also high concentrations of phenolics compounds, the major being derivatives of caffeic acid, apigenin, quercetin and kampherol [12].

Thus, the aim of this work was to evaluate the effect of the addition of dry powder from three underutilised edible plants, fruits of *Rosa canina*, corn of *Quercus ballota* and shoots and leaves of *Sanguisorba minor* to sunflower, seed and olive oils, on lipid oxidation during the frying process.

MATERIALS AND METHODS

Wild edible plants (WEP) and vegetable oils

Fruits of *Rosa canina* L., acorns of *Quercus ballota* Desf. and leaves of *Sanguisorba minor* Scop. were harvested at their optimum stage for consumption according to traditional practices and transferred to laboratory. Then, 3 homogeneous samples (50 g) were randomly performed for each species, which were frozen in liquid N₂, lyophilized and ground to be used as additives to vegetable oils. The vegetable oils used were: virgin olive oil from oil press of Jaen (Spain), sunflower oil (Savena-Ibérica, Sevilla Spain) and special seed oil to frying with increased oleic acid content and E-900 antifoam (Hacendado, Mercadona, Spain). Samples of lyophilized WEP were added to the vegetable oils at 0.5, 1 and 5 % (w:v) and the vegetable oils were heated at 100 °C for 12 h. Experiments were performed in triplicate

Lipid Peroxidation Measurement

Malondialdehyde (MDA) was quantified in duplicate in vegetable oils after the heating process as index of lipid peroxidation using the thiobarbituric acid reactive substrates (TBARS) assay [13]. Reaction mixture contained 1.5 mL 0.5% thiobarbituric acid (TBA) in 20% TCA and 0.5 mL of vegetable oil. The mixture was incubated at 90 °C in a shaking water bath for 20 min, and the reaction was stopped by placing the reaction tubes in an ice-water bath. The samples were then centrifuged at 1000 g for 5 min, and the absorbance of supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mM cm⁻¹ and expressed as mmol MDA g⁻¹ of vegetable oil. The data are the means±SE of quantifications made in duplicated in three replicates.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were calculated using the statistical software (SPSS, version 14.0, SPSS Inc., Chicago, USA). Least significant difference values (LSD; P≤0.05) were calculated to find significant differences among treatments.

Results and Discussion

Peroxides and hydroperoxides are products that represent the beginning of lipoxidation, a step in which the chain-breaker antioxidants can interrupt the process and restore the fatty acid chain [14]. Then, hydroperoxides are spontaneously decomposed to MDA, which can be used as an indicator of lipid peroxidation damage [15]. MDA concentration increased in vegetable oils after the heating process, with important differences among the vegetable oil, and the addition of WEP showed different effect depending on plant species and vegetable oils (**Figure 1**). Thus, in sunflower oil the concentration of MDA increased from initial values of 2.24±0.30 mmol g⁻¹ to 6.53±1.63

mmol g⁻¹ after the heating process, this increase being significantly reduced by the addition of the three WEP. Moreover, this effect was similar for the three plant species and the three used concentrations (**Figure 1 A**) and could be attributed to the high phenolics content of these plants [8-11]. Accordingly, the addition of nigella seed extract to this vegetable oil increased its oxidative stability [5]. Similar effects have been reported in soybean oil enriched with white tea and olive leaves extract, in which the oxidative extension during microwave heating was reduced [16].

However, in olive and seed oils the heating process had no significant effect on MDA content (**Figure 1 A and B**), probably due to the high natural antioxidant compounds present in virgin olive oil, the most important being phenolic compounds (phenolic alcohols, and acids, flavonoids, lignans and secoiridoids), carotenes and tocopherols [17-18] and to the synthetic antioxidant added to the seed oil special to frying. Moreover, the addition of these WEP powders to olive oil was effective on reducing lipid peroxidation when added at 5 %, while at doses 0.5 and 1% no significant effect was observed. Accordingly, the incorporation of rosemary, thyme and lemon dried plants to olive oil just relatively helped to improve its thermal resistance [19]. On the contrary, in seed oil the addition of WEP lead to different effect depending on plant species. Thus, MDA content decreased after the heating process with the addition of *Quercus ballota* corn, while the addition of increased concentration of *Rosa canina* fruit and leaves and shoots of *Sanguisorba minor* led to increases in MDA concentration, showing a pro-oxidant effect (**Figure 1C**). Other antioxidants have also different effects when added to different oils. Thus, TBHQ showed antioxidant effect in sunflower and soybean oils, while it displayed a pro-oxidant effect in corn oil [6].

Results show that the addition of dry powder of wild edible plants with high concentration of natural antioxidants could be an innovative and safety tool to increase thermal stability of vegetable oils such as sunflower, soybean or corn and avoid or decrease the use of synthetic antioxidant.

Conflict of Interests

The authors declare that they have no conflict of interest.

Figure 1: Lipid peroxidation value, expressed as malondialdehyde (MAD) concentration, in vegetable oils before the heating process (control) and after heating (AH) and after the heating process of these edible oils with the addition of several concentration of the wild edible plants (WEP). For each vegetable oil (A: sunflower, B: olive and C: seed oil) data are the mean $\pm$ SE of two determinations performed in three replicates and LSD values are shown.

REFERENCES

1. Guillén, M.D., Goicoechea, E., Toxic oxygenated α , β unsaturated aldehydes and their study in foods: A review, *Critical Reviews in Food Science and Nutrition*, 48 (2008) 119-136.
2. Qin, L.X., Chao, J., Yan, S.Y., Li, Y.M., Gang, C.X., Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS, *Food Chem.* 113 (2009) 692-700.
3. Frankel, E. D., *Antioxidants in Food and Biology. Facts and Fiction*, The Oily Press: Bridgwater, England, 2007.
4. European Official Bulletin, BOE (Order of 24 August 2007). BOE-A-2007-16339, http://www.boe.es/aeboe/consultas/bases_datos/doc.php?id=-
5. Ammari, F., Cordella, C. B. Y., Boughanmi, N., Rutledge, D.N., The increase in oxidative stability of sunflower oil enriched with *Nigella sativa* L. seed extracts, *Food Measure.* 6 (2012) 12-20.
6. Cordeiro, A.M.T.M., Medeiros, M.L., Santos, N.A., Soledade, L.E.B., Pontes, L.F.B.L., Souza, A.L., Queiroz, N., Souza, A.G., Rosemary (*Rosmarinus officinalis* L.) extract - Thermal study and evaluation of the antioxidant effect on vegetable oils, *Journal of Thermal Analysis and Calorimetry* (2012) 1-7. Article in Press.
7. Asensio, C.M., Nepote, V. and Grosso, N.R., Chemical stability of extra-virgin olive oil added with oregano essential oil, *Journal of Food Science*, 76 (2011) 445-450.
8. Barros, L., Carvalho, A.M., Ferreira, I.C.F.R., Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal, *Food Research International*, 44 (2011) 2233-2236.
9. Egea I, Sánchez-Bel P, Romojaro F, Pretel MT. Six edible wild fruits as potential antioxidant additives or nutritional supplements, *Plant Foods for Human Nutrition*, 65 (2010) 121-129.
10. Romojaro, A., Botella, MA., Obón, C. and Pretel, M.T. Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet, *International Journal of Food Science and Nutrition*, (2013). DOI: 10.3109/09637486.2013.821695.
11. Cantos, E., Espín, J.C., López-Bote, C., De la Hoz, L.D., Ordóñez, J.A., Tomás-Barberán, F.A., Phenolic compounds and fatty acids from acorns (*Quercus spp.*), the main dietary constituent of free-range Iberian pigs, *Journal of Agricultural and Food Chemistry*, 51 (2003) 6248-6255.
12. Gatto, M.A., Ippolito, A., Linsalata, V., Cascarano, N.A., Nigro, F., Vanadia, S., Di Venere, D., Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables, *Postharvest Biology and Technology*, 61 (2011) 72-82.
13. Heath, R.L. and Packer, L., Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125 (1968) 189-198.
14. Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability. *Chem Soc Rev.* 39 (2010) 4067-4079.

15. Rogiers, S.Y., Kumar, G.N.M., Knowles, N.R., Maturation and ripening of fruit of *Amelanchier alnifolia* Nutt. are accompanied by increasing oxidative stress, *Annals of Botany*, 81 (1998) 203-211.
16. Malheiro, R., Rodrigues, N., Manzke, G., Bento, A., Pereira, J.A., Casal, S., The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating. *Industrial Crops and Products*, 44 (2013) 37-43.
17. García-González, D.L., Aparicio-Ruiz, R., Aparicio, R., Virgin olive oil - Chemical implications on quality and health. *European Journal of Lipid Science and Technology* 110 (2008) 602-607.
18. Servili, M., Esposto, S., Fabiani, R., Urbani, S., Taticchi, A., Mariucci, F., Selvaggini, R., Montedoro, G.F., Review: Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure, *Inflammopharmacology*, 17 (2009) 76 84.
19. Ayadi, M.A., Grati-Kamoun, N., Attia, H., Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants, *Food and Chemical Toxicology*, 47 (2009) 2613-2619.

Conclusions

1. Traditional orange ready to eat

The best condition to obtain orange segments (*Citrus sinensis* cv. Cadenera) by enzymatic peeling was: 1 ml L⁻¹ of Peelzym II, applied at 53 kPa with three vacuum pulses of two minutes and a subsequent period of 30 minutes in the enzymatic solution at atmospheric pressure. The enzymatic peeling did not affect the antioxidant capacity of the segments, reduced the microbial flora and increased the orange colour. The segments packaged in atmospheres slightly enriched with CO₂ (0.2 -0.7 mL 100 mL⁻¹) had a shelf life of 7 days, because after 10 days of storage, and especially with higher levels of CO₂ (1.17±0.31 mL 100 mL⁻¹), the antioxidant capacity of the segments significantly decreased, the weight loss increased, the microbial contamination increased and loss of aroma occurred.

2. Nutritional and antioxidant properties of wild edible plants

Taking into account both total antioxidant activity and the phenolic content of the plants analysed, *Sanguisorba minor*, *Quercus ballota*, *Sedum sediforme*, and *Cistus albidus* could be used as nutritional supplements in functional foods with high antioxidant activity and as natural antioxidants to replace synthetic ones. In addition, *S. sediforme* also provides a high amount of calcium to the diet, while *S. minor* and the pink flowers of *C. albidus* would provide colour and enhance the appearance of salads. Both *Mesembryanthemum* species and *Oxalis pes-caprae* would only be recommended in small quantities due to their high oxalic acid content, although they would provide pleasant salty and acidic tastes, respectively. Moreover, these wild plants have a greater mineral content than some cultivated vegetables. Thus, the consumption of the investigated plant species could provide health benefits due to their high antioxidant and nutritional values as well as their sensorial properties. These plants could be used to improve ingredient diversity in modern cuisine.

3. Wild edible plants as potential supplements for beverages

The beverages that result from the addition of wild edible plants with high antioxidant capacity to orange juice or kiwifruit cremogenate are well accepted by consumers, even improving the acceptance of the fresh product, and they improve the content in phenolic compounds and the antioxidant capacity without changing the physico-chemical properties of the matrices. This better acceptance of minimally processed enriched beverages by the consumer was due, to a great extent, to the intensification of the natural colour (in the case of addition of *Rosa canina* to orange juice and *Sanguisorba minor* to kiwifruit cremogenate) and to the extra sweetness provided mainly by *Quercus ballota* and, to a lesser extent, by *Rosa canina* because the addition of

wild edible plants to the matrices increased their content in soluble solids. Beverages minimally processed with a high interest for consumers and with high antioxidant properties have been obtained.

4. Wild edible plants as antioxidants in vegetables oils

The addition of dry powder of wild edible plants with high concentration of natural antioxidants could be an innovative and safety tool to increase thermal stability of vegetable oils such as sunflower, soybean or corn and avoid or decrease the use of synthetic antioxidants.

GENERAL CONCLUSION

The plant genetic resources for food and agriculture are the biological basis of world food security and, however, the agronomic and economic factors are favouring the abandonment of the cultivation of many plant species and varieties usually consumed since ancient times. In this work, possible solutions to this loss of genetic diversity have been studied in order to find new commercial outlets for some underutilized plant species and varieties, paying special attention to the increase of the standard of living of people and the changes in consumers' demands regarding nutritional quality. Ready-to-eat segments of an underutilized variety of orange (*Citrus sinensis* cv. Cadenera) were achieved by a methodology designed for commercial citrus fruits. By other hand, it was found that underutilized wild edible plants could be used as functional ingredients in modern diet and to increase the variety in creative cuisine due to their flavours, aromas and textures as well as to their antioxidant potential, like *Sanguisorba minor*, *Quercus ballota* and *Sedum sediforme*; or due to their mineral content, like *Asparagus acutifolius* and *Malva sylvestris*. Moreover, new beverages using *S. minor*, *Q. ballota* and *Rosa canina* mixed with orange juice or kiwifruit cremogenate were developed, and they were very well accepted by consumers and presented significant antioxidant properties. *S. minor*, *Q. Ballota* and *R. canina* also showed a significant protecting effect against lipid oxidation in edible plant oils, so they could be used as natural antioxidants. Therefore, in this Doctoral Thesis some antioxidant, nutritional and organoleptic properties of wild edible plant species were determined and some fresh-cut products with high functional power were developed. These results have brought new technologies close to underutilized plant species and achieved a mutual feedback.

Annexes

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Obtaining and storage of ready-to-use segments from traditional orange obtained by enzymatic peeling

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Obtaining and storage of ready-to-use segments from traditional orange obtained by enzymatic peeling

P. Sanchez-Bel¹, I. Egea¹, M. Serrano², A. Romojaro¹ and M.T. Pretel²

Abstract

The aim of this study was to optimize all parameters implied in the process of obtaining orange segments as minimally processed product by enzymatic peeling, from the method of segment obtaining to the storage conditions, and also to evaluate two films with different permeability. Enzymatic peeling was carried out by fruit infusion under vacuum conditions in a commercial preparation of pectinases and cellulases (Peelzym II). The best condition to obtain Cadenera segments by enzymatic peeling was 1 mL/L of Peelzym II applied at 53 kPa with three vacuum pulses of 2 min and a subsequent period of 30 min in the enzymatic solution at atmospheric pressure. The segments were packaged in two films with different permeability and they were stored for 10 days at 4 °C. Samplings were carried out after 4, 7 and 10 days of storage. The results showed that the concentration of CO₂ in the less permeable film (PA 120) ranged between 0.7 and 1.2 mL/100 mL, while in the most permeable one (PA 240) was around 0.2 mL/100 mL. Under these conditions, the weight loss was kept at low levels until day 7 of storage, showing a significant increase after 10 days. Colour (a/b) increased with the peeling process and it remained without significant changes during the whole storage period for both films. The antioxidant capacity slightly decreased after 10 days of storage. The microbial flora was reduced after the enzymatic peeling. Both the sensory and the microbiological quality were kept in adequate levels for consumption during 7 days. From this moment, they lost sweetness and aroma and the microbial development increased. Thus, the use of the most permeable film and a maximum storage time of 7 days are recommended to maintain the most quality of the segments.

Keywords

Orange, citrus fruit, enzymatic peeling, minimally processed fruits, ready-to-use, films

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INTRODUCTION

The main lines of innovation in the agri-foodstuffs industry are focused on obtaining foods that preserve their sensory characteristics and bioactive compounds by the use of storage techniques like controlled or modified atmospheres, among others. The storage with high levels of CO₂ and low levels of O₂ (keeping the sensory and nutritional quality) of fruits and vegetables considerably extends the storage period at low temperatures

(Robles-Sánchez et al., 2009). On the other side, the increase in the standard of living of the population has implied a change in the eating habits by increasing the preference of consumers for natural products, without addition of preservatives, with a high quality and easy to prepare. In response to this demand, 'ready-to-use' (RTU) or minimally processed products

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have been developed, that is, fresh fruits and vegetables, washed and cut, ready to be consumed and packaged in modified and cold atmospheres that allows an extension of their shelf life under these conditions (Artés and Artés, 2003). The oranges present ideal conditions for being prepared as RTU products, since their distribution in segments allows the sectioning of the fruit without modifying the integrity of the vesicular structure (Pretel et al., 1997). At present, the removal of both albedo and segment membranes of the citrus fruits is carried out by chemical or mechanical procedures that imply high water expenditure and significant negative impact for the environment (Pretel et al., 1998). Enzymatic peeling is an alternative that could reduce the use of water and the degree of contamination in the obtaining of peeled citrus fruits. This new method to obtain peeled citrus fruit is based on infusion under vacuum conditions of an enzymatic preparation, which degrades the pectic substances present in the cell walls (Pretel et al., 2007b, 2008). Many parameters must be taken into account in order to obtain good peeling effectiveness, such as type of enzymatic preparation and its concentration (Baker and Bruemmer, 1989; Bruemmer, 1981; Soffer and Mannheim, 1994), and the vacuum conditions to allow the entrance of enzymatic solution towards the interior of the mesocarp (Baker and Wicker, 1996; Prakash et al., 2001; Pretel et al., 1997, 2005). Moreover, the effect of the incubation time on peeled fruits quality may also be important (Prakash et al., 2001; Pretel et al., 1998; Rouhana and Mannheim, 1994; Soffer and Mannheim, 1994).

On the other hand, the market sales of RTU fresh vegetables have grown rapidly in recent decades as a result of changes in consumer attitudes, especially consumption of fresh-cut lettuce and carrot due to their use in prepared salads (Rico et al., 2007). However, the marketing of fruits, in particular orange, as RTU is nearly nonexistent because there are not enough scientific studies regarding their elaboration or preservation. It is well known that processing of vegetables promotes a faster physiological deterioration, biochemical changes and microbial degradation of the product even when only slight processing operations are used (O'Beirne and Francis, 2003), which may result in degradation of the colour, texture and flavour (Kabir, 1994; Varoquaux and Wiley, 1994). Reduced O₂ and high CO₂ levels have proven to effectively control enzymatic browning, softening and decay in fresh-cut vegetables (Oms-Oliu et al., 2008), the full beneficial effect of modified atmosphere being attained by correctly choosing the packaging materials (Allende et al., 2006).

This work offers new data about a topic that has not been deeply studied, that is, the obtaining and storage of RTU citrus fruits by non-contaminant methods (enzymatic peeling) using a Spanish traditional

orange variety. Moreover, this is the first work that globally optimises all parameters implied in the process of obtaining orange segments as RTU product, from the process to obtain fruit segments by enzymatic peeling until their storage under atmospheres slightly enriched with CO₂.

MATERIALS AND METHODS

Materials

Plant material. A traditional variety of orange fruit (*Citrus sinensis* (L.) Osbeck cv. Cadenera) grown in the South-east of Spain was used. This variety belongs to the group of common oranges that includes white oranges (Rivera et al., 1998). Fruits were harvested at the optimal ripening stage (average respiratory rate 14.53 ± 2.30 mg CO₂/kg h, ethylene production rate 0.12 ± 0.01 nL/g h, titratable acidity 1.04 ± 0.04 g/100 g and soluble solids content at 20 °C 14.3 ± 1.26 °Brix). The acidity and soluble solids were analysed according to Pretel et al. (1998).

Plastic films. The plastic films employed were provided by the firm Sidlaw Packaging P-Plus. Two microperforated polypropylene plastics with non-selective permeability were employed: Film PA120 (permeability at 25 °C for CO₂ and O₂: 120,000 mL/d m²; thickness: 35 µm) and Film PA240 (permeability at 25 °C for CO₂ and O₂: 240,000 mL/d m²; thickness: 35 µm).

Commercial enzyme preparation. The pectolytic enzyme preparation used, provided by the company Novo Nordisk Ferment Ltd (Dittingen, Switzerland), was Peelzym II produced by *Aspergillus niger*. This enzymatic product is very suitable for the degradation of albedo and carpelar membrane due to its high pectinase and polygalacturonase activities (Pretel et al., 2005).

Determination of ideal peeling conditions

Determination of the best combination of vacuum pressure and application time. Potential enzymatic saturation of albedo (PESA) was considered as the amount of enzymatic solution that the albedo fruit would be able to absorb. To evaluate PESA, oranges without any visible external damage were placed in a water bath at 40 °C to reach the recommended temperature for enzymatic peeling (Pretel et al., 1997; Soffer and Mannheim, 1994). The entire fruit surface was homogeneously perforated by rolling the oranges over a 1 m² wood plate provided with cylindrical metal parts of 5 mm long and 1 mm diameter. The distance between each cylindrical metal part was 10 mm. With this system 8 ± 3 perforations per cm² in the albedo fruit

were produced. After perforation, the fruits were placed in a vacuum tank containing 9 L of the Peelzym II solution (1 mL/L) with Chinese ink (1:10) at 40 °C and submitted to different vacuum pressures, 27, 40, 53, 67, 80 and 93 kPa during different application times (pulses of 2 min). The PESA was measured using two parameters: percentage of fruit weight increase and percentage of the albedo surface dyed with ink. In all experiments, 10 fruits were taken for each vacuum pressure and application time according to Pretel et al. (2007b). From this experiment, the best combination of vacuum pressure and application time was selected.

Optimization of the incubation time for enzymatic peeling process. Peelzym II concentration at 1 or 2 mL/L were used according to the previous experiments. A total of 60 orange fruits (30 for each concentration of Peelzym II) without any visible external damage were washed with chlorinated water (300 ppm), placed in a water bath at 40 °C and perforated as previously described. Then, orange fruits were exposed to the vacuum conditions previously optimised and thereafter vacuum was released and orange fruits were left to incubate with the enzymatic solution at atmospheric pressure. Samplings of five fruit were undertaken after six different incubation times (10, 20, 30, 40, 50 and 60 min) and the peeling efficiency was evaluated, according to five visual parameters dealing the final product quality. Since these parameters were subjective, each of them was independently assessed by five judges for each of the five fruits of each experiment. The quality parameter evaluated and scores for them were the following (Pretel et al., 2007b):

- Percentage of albedo not attacked: an estimation of the percentage of the fruit surface not attacked by the enzymatic solution.
- Ease of skin removing (albedo + flavedo): from very easy (5) to very difficult (1).
- Fruit firmness: from high (5) to low (1).
- Ease of segment separation: from very easy (5) to very difficult (1).
- Percentage of commercial viable segments: That is, percentage of segments without defects.

Enzymatic peeling and sample preparation.

Enzymatic peeling was carried out by vacuum infusion of an enzymatic solution of Peelzym II (1 mL/L) applied at 53 kPa with three vacuum pulses of 2 min, 40 °C to remove the peel, and a subsequent period of 30 min in the enzymatic solution at atmospheric pressure (these were the best conditions for obtaining orange segments according to the results of the experiments described above). Then, fruits were rinsed by

Figure 1. Increase in weight of fruits (a) and albedo surface dyed with ink (b) of fruits of the Cadenera variety after applying different vacuum pressures (27, 40, 53, 80 and 93 kPa) for different times: 2 min (2min), 4 min in two pulses (2+2min) and 6 min in three pulses (2+2+2min). Data are the mean $\pm$ SE of 10 fruits and the LSD_{0.05} value is shown. (○) 27 kPa, (□) 40 kPa, (△) 53 kPa, (●) 67 kPa, (■) 80 kPa and (▲) 93 kPa.

immersion in water for 30 min at 4 °C. The segments were withdrawn and manually centrifuged to remove water from their surfaces and then packaged. Different plastic bags (interchange surface 1000 cm²) were filled with approximately 300 g of oranges and each was fitted with a silicon septum used for sampling the internal atmosphere to analyse gas composition. All samples were stored during 10 days at 4 $\pm$ 1 °C, the temperature normally used in the commercialization of these products. Variation of the atmosphere

Table 1. Peeling efficiency of fruits at different incubation times with 1 and 2 mL/L of Peelzym II

Incubation time (min)	Peelzym II (mL/L)	Albedo not attacked ¹ (%)	Ease of skin separation ¹	Ease of segment separation ¹	Segment firmness ²	Commercial viable segments ² (%)
10	1	17.7 ± 3.20 a	3.7 ± 0.6 a	4.67 ± 0.47 a	4.0 ± 0.71 a	90 ± 6.14a
	2	8.34 ± 2.36 a	5 ± 0 b	4.12 ± 0.24 a	4.17 ± 0.24 a	86.67 ± 9.43 a
20	1	1.31 ± 1.36 b	5 ± 0 b	4.84 ± 0.24 a	4.0 ± 0.41 a	100 ± 5.1 a
	2	2.84 ± 1.18 b	4.7 ± 0.5 b	3.84 ± 0.47 b	3.84 ± 0.85 a	85.34 ± 7 a
30	1	3.34 ± 2.36 b	5 ± 0 b	4.67 ± 0.47 a	4.67 ± 0.24 a	100 ± 0 a
	2	3.30 ± 0.8 b	5 ± 0 b	3.84 ± 0.43 b	3.67 ± 0.17 a	96.67 ± 4.7 a
40	1	2.34 ± 2.05 b	5 ± 0 b	3.50 ± 0.41 b	4.01 ± 0.71 a	100 ± 3.2 a
	2	1.50 ± 1.08 b	5 ± 0 b	4.0 ± 0.39 a	3.34 ± 0.47 a	93.34 ± 4.71 a
50	1	1.67 ± 2.36 b	5 ± 0 b	2.67 ± 0.24 c	3.17 ± 1.03 a	76.67 ± 4.25 a
	2	1.67 ± 1.91 b	5 ± 0 b	4.0 ± 0.41 a	3.84 ± 0.85 a	66.67 ± 4.71 b
60	1	0.92 ± 0.02 b	5 ± 0 b	3.17 ± 0.24 b	3.34 ± 0.24 a	73.34 ± 4.43 a
	2	0.67 ± 0.94 b	5 ± 0 b	3.67 ± 0.45 b	3.0 ± 0.82 a	63.34 ± 3.57 b

Note: Values within a row followed by different letters are significantly different ($p < 0.05$).

¹Data are the mean ± SE of evaluations made independently in five fruits.

²Data are the mean ± SE of evaluations made independently in five segments.

composition inside the bags was checked daily. Periodic samplings were carried out on three trays of each film at 4, 7 and 10 days of storage in which weight loss, colour, sensory quality, antioxidant capacity and microbiological and sensory quality of segments were analysed. Some parameters like colour and microbial content were also analysed before peeling (BP) and after peeling (AP) in order to know if, apart from the preservation method, the peeling process has any effect on the quality of segments.

Evaluation of the quality of segments during storage

Gas analysis inside packages. A silicone septum was located on each bag's surface in order to enable sampling gas inside each package. In all, 1 mL of the bag headspace atmosphere was withdrawn using an airtight syringe and the CO₂ and O₂ concentrations were measured by gas chromatography with a GC 14B (Shimadzu, Tokyo, Japan), equipped with a thermal conductivity detector (TCD) and a molecular sieve 5A column, 80–100 mesh (Carbosieve SII, Supelco Inc., Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperature were 50 °C and 110 °C, respectively. Helium was used as the carrier gas with a flow rate of 50 mL/min. Results are expressed as mL/100 mL of O₂ and CO₂ inside the bags and are the mean ± SE of duplicate determinations in each bag ($n = 5$). A further 1 mL of the bag atmosphere was used to quantify ethylene concentration using a Hewlett-Packard gas chromatograph model 5890 (Wilmington, DE, USA)

equipped with a flame ionisation detector and a stainless steel column packed with 80/100 mesh-activated alumina. Oven temperature was 90 °C and injector and detector temperatures 150 °C. N₂ was used as the carrier gas at a flow rate of 28 mL/min. Results are expressed as µL/L inside the bags and are the mean ± SE of duplicate determinations in each bag ($n = 5$).

Weight loss and colour. Citrus segment were weighted before packaging and after the storage period, and weight loss was calculated as percentage of their initial weight ($n = 3$). Colour was determined using a reflection colorimeter Hunter model D-25A-2 with 45° illumination, which expresses colour in coordinates L*, a* and b*. Two measurements were made at opposite sides of each fruit segment and results represent the mean ± SE of the measurements taken from 10 fruit segments of each bag.

Sensory evaluation. Sensory analysis to compare the quality of orange segments was carried out by 10 trained adults, aged 25–40 (5 female and 5 male). The panel was trained in a pretest in which orange fruit with extremely low or high attributes (aroma, lack of bitterness, chewiness and segment firmness) were evaluated (Pretel et al., 1998). A laboratory of sensory analyses with an individual booth for each panellist was used. Panellists were randomly served five segments from each film for each variety to evaluate the above mentioned attributes on a ranked scale of 1 to 5. The selection and training of tasters, as well as the fitting-out of

Figure 2. Evolution of the internal atmosphere (a) mL/100 mL CO₂, (b) mL/100 mL O₂ and (c) µL/L C₂H₄ during 10 days of storage at 4 °C inside the bags made of films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five bags. LSD_{0.05} value is shown. (●) PA 120 and (▼) PA 240.

the tasting room, were carried out according to AENOR (1995).

Determination of total antioxidant activity. Total antioxidant activity (TAA) was determined using the enzymatic system composed of the chromophore 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which ABTS^{•+} radicals are generated.

After that, the vegetable extracts are added and their antioxidant compounds regenerate the ABTS (Cano et al., 1998; Pretel et al., 2004). The reaction mixture contained 1.5 mM ABTS, 15 µM hydrogen peroxide and 0.25 µM HRP in 50 mM glycine-HCl buffer (pH 4.5) in a total volume of 2 mL. The assay temperature was 25 °C and the reaction was monitored at 414 nm, until a stable absorbance was obtained using a UNICAM Helios α spectrophotometer (Cambridge, UK). After that, a suitable amount of orange extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with [®]-(+)-6-hydroxy-2, 5, 7, 8-tetramethylchoman-2-carboxylic acid (Trolox; 0–20 nmol) from Sigma (Poole, Dorset, UK), and results are expressed as milligram of Trolox equivalent per 100 g of fresh weight (mg/100 g).

Microbiological analyses. Microbiological growth during the storage of samples was determined by total plate count on plate count agar (PCA, Oxoid), by *Lactobacillus* count on MRS agar (Oxoid) and by yeast and mould count on Rose Bengal chloramphenicol agar (Oxoid). Samples were examined on days 3, 7 and 10. Three bags were opened under sterile conditions and a 10 g sample was placed into a stomacher bag with 90 mL of peptone water (Oxoid). Samples were homogenized in peptone water. Appropriate dilutions were placed on two duplicate plates of PCA, MRS or Rose Bengal medium. Plates were incubated at 30 °C for 48–72 h and counts were obtained.

Statistical analysis. Data for the physical, chemical and visual quality parameters were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatments. Mean comparisons were performed using the honestly significant difference Tukey's test to examine if differences between plastic films and storage time were significant at $p < 0.05$. All analyses were performed with a statistical software package SPSS v. 11.0 for Windows least significant differences (LSDs) ($p = 0.05$) were calculated for mean separations and are showed in figures.

RESULTS AND DISCUSSION

Ideal peeling conditions

Different vacuum conditions were applied to orange fruit to determine the minimum vacuum pressure and the minimum number of pulses to be applied to the fruits to allow the entrance of an adequate amount of the enzymatic solution in the albedo and between the segments of the Cadenera orange. A total saturation of the enzymatic solution in the albedo was achieved with pressures between 53 and 93 kPa applied in three vacuum pulses of 2 min, as it can be deduced from

Figure 3. Evolution of the weight loss of 'Cadenera' orange segments obtained by enzymatic peeling during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made from three different trays from each bag. LSD_{0.05} value is shown. (■) PA 120 and (▨) PA 240.

Figure 4. Evolution of colour (a^*/b^*) of 'Cadenera' orange segments obtained by enzymatic peeling, before peeling (BP), after peeling (AP) and during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five segments from three different trays from each bag. LSD_{0.05} value is shown. (▩) BP, (▤) AP, (■) PA 120 and (▨) PA 240.

the results of the PESA studies (Figure 1(a) and (b)). In order to obtain segments, it is important to determine the vacuum conditions at which the fruit medulla begins to be dyed. This would indicate the penetration of solution inside the fruit and its separation into individual segments (Baker and Bruemmer, 1989; Pretel et al., 2007a). The vacuum pressures with which the medulla was dyed began from 53 kPa and three vacuum pulses. Therefore, to obtain segments from Cadenera variety, the best vacuum conditions are 53 kPa with three

Figure 5. Evolution of the total antioxidant activity (TAA) of 'Cadenera' orange segments obtained by enzymatic peeling, before peeling (BP), after peeling (AP) and during 10 days of storage at 4 °C in the films PA 120 and PA 240. Each data was represented as the mean $\pm$ SE of the measurements made in duplicate in five segments from three different trays from each bag. LSD_{0.05} value is shown. (▩) BP, (▤) AP, (■) PA 120 and (▨) PA 240.

vacuum pulses. The use of 67–93 kPa would not be recommended because an excess of enzyme solution in tissue as porous as the albedo could be a disadvantage for the process of enzymatic peeling, in which the infiltrated material cannot be used again (Baker and Wicker, 1996).

On the other hand, it was shown that the effectiveness of the process varied greatly depending on the concentration of the enzyme used (Bruemmer, 1981; Pretel et al., 1997; Toker and Bayindirli, 2003). The enzymatic solutions are expensive and it is possible to reduce the amount of enzymatic solution by increasing the incubation time (Prakash et al., 2001). The highest albedo percentage not attacked by the enzyme was found after 10 min of incubation with 1 mL/L of Peelzym II ($17.7 \pm 3.2\%$; Table 1), which would decrease the quality of the final product (Pretel et al., 2007a). However, after 20 min, the percentage of albedo not attacked was less than 3%, a percentage that allows a very good peeling efficiency. The ease of skin separation was reached from 10 min of incubation with 2 mL/L of enzyme preparation, while with 1 mL/L, the maximum ease of skin was reached after 20 min of incubation. The degree of segment separation with 1 mL/L of enzyme preparation has its optimum after 30 min incubation time (4.67 ± 0.47) with a percentage of albedo not attacked of $3.34 \pm 2.36\%$. With 2 mL/L and more than 30 min incubation time, the segments separation did not greatly differ. Prakash et al. (2001) found that for the enzymatic peeling of grapefruit a minimum of 12 min of incubation was needed to obtain a good ease of peeling, while with

Table 2. Sensory analysis (sweetness, aroma, chewiness and firmness) of 'Cadenera' orange segments obtained by enzymatic peeling during 10 days of storage at 4 °C in the films PA 120 and PA 240

Film	Parameter	Day 0	Day 4	Day 7
PA 120	Sweetness	3.08 ± 0.76 a	3.54 ± 0.62 a	2.52 ± 0.31 a
	Aroma	2.92 ± 0.49 a	2.71 ± 0.31 a	1.51 ± 0.22 b
	Chewiness	4.31 ± 0.8 5a	4.40 ± 0.14 a	4.92 ± 0.32 a
	Firmness	2.35 ± 0.69 a	2.92 ± 0.81 a	4.84 ± 0.33 b
PA 240	Sweetness	3.08 ± 0.76 a	3.05 ± 0.04 a	3.02 ± 0.21 a
	Aroma	2.92 ± 0.49 a	3.28 ± 0.62 a	2.21 ± 0.14 b
	Chewiness	4.31 ± 0.85 a	4.37 ± 0.11 a	4.8 ± 0.51 a
	Firmness	2.35 ± 0.69 a	3.06 ± 0.64 a	4.9 ± 0.21 b

Note: Data are the mean ± SE of determinations made in five segments stored in different films by five judges. Values within a row followed by different letters are significantly different ($p < 0.05$).

incubation times above 30 min, the segments texture was damaged. Therefore, 30 min could be considered as the best time for obtaining peeled segments from Cadenera variety, and the best Peelzym II concentration was 1 mL/L. With 20 min of incubation, good results were also obtained but increasing the concentration of Peelzym II up to 2 mL/L.

Quality of segments during storage

Development of the atmosphere in the different films. The evolution of the internal atmosphere in the bags containing the orange segments is shown in Figure 2. An increase of CO₂ (Figure 2(a)) and decrease of O₂ (Figure 2(b)) took place within both films. The composition of the atmosphere in the less permeable film (PA 120) was around 0.7 mL/100 mL of CO₂ and 21 mL/100 mL of O₂ during the first 7 days, although 1.2 mL/100 mL of CO₂ was achieved after 10 days of storage. In the most permeable film (PA 240), the concentration of CO₂ was 0.2 mL/100 mL during the whole experience, and the concentration of O₂ was kept in the same levels than the film PA 120, that is, around 21 mL/100 mL. In both films, especially in the most permeable PA 240, the concentrations of CO₂ were low and the oxygen was almost not modified compared to the normal air. Therefore, although both films modified the atmosphere, the O₂ was less affected than CO₂ by films. Thus, development of off flavours or undesirable effects could not be expected, since they usually appear when high CO₂ and low O₂ concentration are achieved inside film (Del-Valle et al., 2009; Pretel et al., 1998). However, the packaging plays a significant role in order to prevent dehydration and to maintain texture (Brecht et al., 2003). Moreover, it has been shown in table grape that the atmospheres slightly CO₂ enriched can affect some quality parameters like reducing the weight loss or delaying the microbial development (Pretel et al.,

2006). The ethylene level inside the bags was very low in both films during the whole storage period (under 0.06 µL/L; Figure 2(c)).

Evolution of the weight loss, colour, antioxidant capacity and sensory quality. Consumers consider the aspect of products as a primary factor of valuation, and texture and colour are the main attributes for considering the freshness of the vegetables (Kays, 1999). The potential of MAP to extend shelf life for many foods has been well documented (Brecht et al., 2003; Jacxsens et al., 2001; Saltveit, 2003). Weight loss (Figure 3) increased as storage period extended reaching final levels around 0.5 g/100 g. In addition, weight loss was lower in segments inside the less permeable film compared to the more permeable, during the first 7 days of storage.

On the other side, the characteristic colour of the segments is defined by a mixture of red (+a*) and yellow (+b*), so the a*/b* coefficient gives an idea of the degree in which the red and yellow colours contribute to the orange colour typical of these fruits due to the accumulation of different carotenoids. There are evidences that show that the ratio between yellow carotenoids and orange carotenoids is a good indicator of the characteristic colour of each variety (Alquézar et al., 2008). Therefore, a high a*/b* value should indicate a more intense orange colour, while a low a*/b* value should indicate a loss of the characteristic colour. There were slight changes in colour during storage, and no differences between films were observed (Figure 4). Thus, storage in modified atmospheres kept the initial colour of the segments, as it has been observed in other orange fruits like mangoes (Pesis et al., 2000). However, after the process of peeling, the segments showed significantly higher values ($p < 0.05$) of the parameter a/b (-0.18 ± 0.002) than BP (-0.21 ± 0.010). This means that the process of enzymatic peeling increased the

orange colour of segments, so they were more attractive for the consumer; probably due to the fact that the enzymes employed during the process remove the rests of albedo and skin that can hide the colour of the segments before the enzymatic peeling.

The TAA of vegetables depends on many bioactive phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments (Oms-Oliu et al., 2008). It has been shown that the use of modified atmospheres is a good method for preserving the antioxidant capacity in vegetables (Murcia et al., 2009). Orange juice is an important source of carotenoids and ascorbic acid, valuable for its vitamin action, antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported (Cortés et al., 2008). TAA in orange segment did not change after the enzymatic peeling or after the first 7 days of storage. However, after 10 days, a significant decrease on TAA occurred in segment under film PA 120, while no changes were found in those under film PA 240 (Figure 5). Other studies have shown that the antioxidant capacity of juices treated with high hydrostatic pressure (HHP) and cold stored at $4 \pm 2^\circ\text{C}$ during 21 days did not change (Fernández García et al., 2001). In general, most authors coincide with our results, and the antioxidant capacity does not change during storage (Alasalvar et al., 2005; Murcia et al., 2009; Robles-Sánchez et al., 2009), although sometimes the decrease of C vitamin could explain the decrease of the antioxidant capacity of some vegetables stored under modified atmosphere (Villanueva et al., 2005).

The segments, in both films, were kept in optimum conditions during the first 4 days (Table 2). For both films, changes along storage time in sweetness and chewiness were not statistically significant ($p < 0.05\%$). However, a decrease in aroma was found after 7 days of storage in both plastic films. In addition, a significant increase in segment firmness was found after 7 days of storage, irrespectively of the film used, probably due to a rigidification of the segment surface caused by dehydration processes leading to increases in weight loss. These results state that the enzymatically peeled orange segments can be stored in atmospheres slightly enriched in CO_2 (between 0.2 mL/100 mL and 0.7 mL/100 mL) for a week without loss of sensory quality, because the atmospheres with high levels of CO_2 cause higher losses of aroma and flavour, as was shown in manually peeled orange segments under modified atmospheres (Pretel et al., 2008).

Influence of the enzymatic peeling and the film permeability on the microbial development. The use of low temperatures is one of the main microbiological control methods, but some authors have mentioned that atmospheres with high CO_2 and low O_2 also delay the

Figure 6. Evolution of (A) mesophilic aerobic microflora, (B) moulds and yeasts and (C) lactic microflora before peeling (BP), after peeling (AP) and during 10 days of storage at 4°C in the films PA 120 and PA 240. The a and b represent the homogeneous sub-groups generated by Tukey's test, using the film as factor, at the 95% confidence level ($p \leq 0.05$). (▨) BP, (▤) AP, (■) PA 120 and (▩) PA 240.

microbial development (Carlin et al., 1990). It is interesting to mention the decrease of the microbial development (Figure 6), especially of moulds and yeasts (Figure 6(b)) after the peeling process, probably due to the fact that the enzymes employed in the peeling also have a fungicide or bactericide effect (Pinnavaia et al., 2006; Pretel et al., 1998). However, it was possible that the enzyme peeling removes excess peel, which may harbor bacteria, and this may explain the decrease in

bacterial count AP. The mesophilic aerobic microflora (Figure 6(a)) remained in acceptable levels close to the initial ones up to day 7 of storage with few differences between both films, although it was always slightly higher in the most permeable film. After a week of storage, the development of mesophilic flora experienced a considerable increase so the segments were not commercially viable. This can be due to the fact that the CO₂ concentration in the internal atmosphere of the bags is not high enough for stopping the development of the mesophilic flora, as was observed in manually separated orange segments with CO₂ concentrations over 5 mL/100 mL (Pretel et al., 1998). The evolution of the content of moulds and yeasts (Figure 6(b)) showed a similar behaviour, with levels close to the initial ones up to day 4 in the less permeable film. The CO₂ concentrations reached inside the bags (0.7 mL/100 mL) could probably have a fungistatic effect, as it has been suggested by some authors (Carlin et al., 1990). From day 7 in the film PA 120 and from day 4 in the film PA 240, the levels of moulds and yeasts increased. The lactic microflora (Figure 6(c)) remained close to the initial values during the first week of storage in both films and then considerably increased after 10 days. The differences between the films were not significant. However, one of the main concerns about the use of treatments for extending the shelf life of RTU products associated to the use of high levels of CO₂ and low levels of O₂ is that these treatments can create favourable environments for the development of pathogenic bacteria (Rico et al., 2007; Rosnes et al., 2003).

CONCLUSION

The best condition to obtain Cadenera segments by enzymatic peeling was: 1 mL/L of Peelzym II, applied at 53 kPa with three vacuum pulses of 2 min and a subsequent period of 30 min in the enzymatic solution at atmospheric pressure. The enzymatic peeling did not affect the antioxidant capacity of the segments, reduced the microbial flora and increased the orange colour. The segments packaged in atmospheres slightly enriched with CO₂ (0.2–0.7 mL/100 mL) had a shelf life of 7 days, because after 10 days of storage, and especially with higher levels of CO₂ (1.17 ± 0.31 mL/100 mL), the antioxidant capacity of the segments significantly decreased, the weight of loss increased, the microbial contamination increased and loss of aroma occurred.

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References

- AENOR (1995). *Análisis Sensorial. Guía para la Instalación de una Sala de Cata*. UNE 87 004 79. Asociación Española de Normalización y Certificación, Madrid, España: Asociación Española de Normalización y Certificación.
- Alasalvar C, Al Farsi M, Quantick PC, Shahidi F and Wiktorowicz R. (2005). Effect of chilled storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready to eat shredded orange and purple carrots. *Food Chemistry* 89(1): 69–76.
- Allende A, Tomás Barberán FA and Gil I. (2006). Minimal processing for healthy traditional foods. *Trends in Food Science and Technology* 17(9): 513–519.
- Alquézar B, Rodrigo MJ and Zacarias L. (2008). Regulation of carotenoid biosynthesis during fruit maturation in the red fleshed orange mutant Cara Cara. *Phytochemistry* 69(10): 1997–2007.
- Artés F and Artés F (2003). Etapas decisivas y diseño de instalaciones para elaboración de productos en fresco. In: Lobo G and González M (eds) *Productos Hortofrutícolas Mínimamente Procesados*. Canarias: Consejería de Agricultura, Ganadería, Pesca y Alimentación, pp.92.
- Baker RA and Bruemmer JH. (1989). Quality and stability of enzymatically peeled and sectioned citrus fruit. In: Jen JJ (ed.) *Quality Factors of Fruits and Vegetables*. Washington, DC: American Chemical Society, 140–148.
- Baker RA and Wicker L. (1996). Current and potential applications of enzyme infusion in the food industry. *Trends in Food Science and Technology* 7(9): 279–284.
- Brecht JK, Chau KV, Fonseca SC and Oliviera FAR. (2003). CA transport of fresh produce in MAP: designing systems for optimal atmosphere conditions throughout the post harvest handling chain. *Acta Horticulturae* 600: 799–801.
- Bruemmer JH (1981). *Method of Preparing Citrus Fruit Sections with Fresh Fruit Flavor and Appearance*. US Patent Number 4.284.651.
- Cano A, Hernández Ruiz J, García Cánovas F, Acosta M and Arnao MB. (1998). An end point method for estimation of the total antioxidant activity in plant material. *Phytochemical Analysis* 9(4): 196–202.
- Carlin F, Nguyen The C and Chambroy Y. (1990). Effects of controlled atmospheres on microbial spoilage, electrolyte leakage and sugar content of fresh 'ready to eat' grated carrots. *International Journal of Food Science and Technology* 25: 110–119.
- Cortés C, Esteve MJ and Frígola A. (2008). Effect of refrigerated storage on ascorbic acid content of orange juice treated by pulsed electric fields and thermal pasteurization. *European Food Research and Technology* 227(2): 629–635.
- Del Valle V, Hernandez Munoz P, Catala R and Gavara R. (2009). Optimization of an equilibrium modified atmosphere packaging (EMAP) for minimally processed mandarin segments. *Journal of Food Engineering* 91(3): 474–481.
- Fernández García A, Butz P and Tauscher B. (2001). Effects of high pressure processing on carotenoid extractability,

- antioxidant activity, glucose diffusion, and water binding of tomato puree (*Lycopersicon sculentum* Mill). *Journal of Food Science* 66(7): 1033–1038.
- Jacxsens L, Devlieghere F and Debevere J. (2001). Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh cut produce. *International Journal of Food Microbiology* 71(2–3): 197–210.
- Kabir H. (1994). Fresh cut vegetables. In: Brods L and Herndon VA (eds) *Modified Atmosphere Food Packaging*. New York, NY: Institute of Packaging Professionals, 155–160.
- Kays SJ. (1999). Preharvest factors affecting appearance. *Postharvest Biology and Technology* 15(3): 233–247.
- Murcia MA, Jiménez Monreal AM, García Díaz L, Carmona M, Maggi L and Martínez Tomé M. (2009). Antioxidant activity of minimally processed (in modified atmospheres), dehydrated and ready to eat vegetables. *Food and Chemical Toxicology* 47(8): 2103–2110.
- O’Beirne D and Francis GA. (2003). Reducing the pathogen risk in MAP prepared produce. In: Ahvenainen R (ed.) *Novel Food Packaging Techniques*. Cambridge; Boca Raton, FL: Woodhead Publishing Limited/CRC Press LLC, 231–286.
- Oms Oliu G, Soliva Fortuny R and Martín Belloso O. (2008). Effect of ripeness on the shelf life of fresh cut melon preserved by modified atmosphere packaging. *European Food Research and Technology* 225(3–4): 301–311.
- Pesis E, Aarón D, Aharon Z, Ben Aire R, Aarón N and Fuchs Y. (2000). Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit. *Postharvest Biology and Technology* 19(1): 93–101.
- Pinnavaia S, Baldwin EA, Plotto A, Narciso J and Senesi E. (2006). Enzyme peeling of Valencia oranges for fresh cut slices. *Proceedings of the Florida State Horticultural Society* 119: 335–339.
- Prakash S, Singhal RS and Kulkarni PR. (2001). Enzymic peeling of Indian grapefruit (*Citrus paradisi*). *Journal of the Science of Food and Agriculture* 81: 1440–1442.
- Pretel MT, Amorós A, Botella MA, Serrano M and Romojaro F. (2005). Study of albedo and carpelar membrane degradation for further application in enzymatic peeling of citrus fruits. *Journal of the Science of Food and Agriculture* 85(1): 86–90.
- Pretel MT, Botella MA, Amorós A, Serrano M, Egea I and Romojaro F. (2007a). Obtaining fruit segments from a traditional orange variety (*Citrus sinensis* (L.) Osbeck cv. Sangrinala) by enzymatic peeling. *European Food Research and Technology* 225(5–6): 783–788.
- Pretel MT, Botella MA, Amorós A, Zapata PJ and Serrano M. (2007b). Optimization of vacuum infusion and incubation time for enzymatic peeling of ‘Thomson’ and ‘Mollar’ oranges. *LWT Food Science and Technology* 40(1): 12–30.
- Pretel MT, Botella MA, Zapata PJ and Serrano M. (2004). Antioxidant activity and general fruit characteristics in different traditional orange (*Citrus sinensis* (L.) Osbeck) varieties. *European Food Research and Technology* 219(5): 474–478.
- Pretel MT, Fernández PS, Martínez A and Romojaro F. (1998). The effect of modified atmosphere packaging on “ready to eat” oranges. *Lebensmittel Wissen Und Technologie* 31(4): 322–328.
- Pretel MT, Lozano P, Riquelme F and Romojaro F. (1997). Pectic enzymes in fresh fruit processing: optimization of enzymic peeling of oranges. *Process Biochemistry* 32(1): 43–49.
- Pretel MT, Martínez Madrid MC, Martínez JR, Carreño JC and Romojaro F. (2006). Prolonged storage of ‘Aledo’ table grapes in a slightly CO₂ enriched atmosphere in combination with generators of SO₂. *LWT Food Science and Technology* 39(10): 1109–1116.
- Pretel MT, Sanchez Bel P, Egea I and Romojaro F. (2008). Enzymatic peeling of citrus fruits: factors affecting degradation of the albedo. In: Teixeira da Silva JA (ed.) *Tree and Forestry Science and Biotechnology*. Isleworth: Global Science Books, 52–59.
- Rico D, Martín Diana AB, Barat JM and Barry Ryan C. (2007). Extending and measuring the quality of fresh cut fruit and vegetables: a review. *Trends in Food Science and Technology* 18(7): 373–386.
- Rivera D, Obón C, Ríos S, Selma C, Méndez F, Verde A, et al. (1998). *Las variedades tradicionales de frutales de la cuenca del río Segura. Catálogo etnobotánico. Cítricos, frutos carnosos y vides*. Murcia: Servicio de Publicaciones de la Universidad de Murcia.
- Robles Sánchez RM, Rojas Graü MA, Odriozola Serrano I, González Aguilar GA and Martín Belloso O. (2009). Effect of minimal processing on bioactive compounds and antioxidant activity of fresh cut ‘Kent’ mango (*Mangifera indica* L.). *Postharvest Biology and Technology* 51(3): 384–390.
- Rosnes JT, Sivertsvik M and Skåra T. (2003). Combining MAP with other preservation techniques. In: Ahvenainen R (ed.) *Novel Food Packaging Techniques*. Cambridge: Woodhead publishing Ltd, 287–311.
- Rouhana A and Mannheim CH. (1994). Optimization of enzymatic peeling of grapefruit. *Lebensmittel Wissen Und Technologie* 27(2): 103–107.
- Saltveit ME. (2003). Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology* 27(1): 3–13.
- Soffer T and Mannheim CH. (1994). Optimization of enzymatic peeling of oranges and pomelo. *Lebensmittel Wissen Und Technologie* 27(3): 245–248.
- Toker I and Bayindirli A. (2003). Enzymatic peeling of apricots, nectarines and peaches. *Lebensmittel Wissenschaft Und Technologie* 36(2): 215–221.
- Varoquaux P and Wiley R. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: Wiley RC (ed.) *Minimally Processed Refrigerated Fruits and Vegetables*. New York: Chapman and Hall, 226–268.
- Villanueva MJ, Tenorio MD, Sagardoy M, Redondo A and Saco MD. (2005). Physical, chemical, histological and microbiological changes in fresh green asparagus (*Asparagus officinalis* L.) stored in modified atmosphere packaging. *Food Chemistry* 91(4): 609–619.

RESEARCH ARTICLE

Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet

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Abstract

Thirteen species of wild edible plants belonging to 11 botanical families consumed in the traditional Mediterranean diet were evaluated. *Sanguisorba minor*, *Quercus ballota* and *Sedum sediforme* showed the highest hydrophilic total antioxidant activity (H TAA) and total phenols. *Asparagus acutifolius*, *Allium ampeloprasum*, *Foeniculum vulgare* and *Malva sylvestris* presented high levels of potassium, *Malva* and *Asparagus* are interesting due to their zinc content, and *Urtica urens* contains a high content of calcium. Sensory analysis indicated that fruits from *Q. ballota* could be considered very sweet and plants of *Crithmum maritimum* and *Oxalis pes caprae* are very acidic. Moreover, testers highlighted the salty taste of *C. maritimum*, *Mesembryanthemum nodiflorum* and *Mesembryanthemum cristalinum*, the spicy taste of *A. ampeloprasum*, and the aroma of *F. vulgare*. Our results indicate that increased consumption of the investigated plant species could provide health benefits. Moreover, due to their sensorial properties, they could be used as new ingredients to improve the diversity in modern diet and highly creative cuisine.

Keywords

Antioxidant activity, modern diet, nutritional properties, wild edible plants

History

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Introduction

The world is presently over dependent on a few plant species. Diversification of production and consumption habits to include a broader range of plant species, particularly those currently identified as under utilised, could significantly contribute to improve health and nutrition, livelihoods and ecological sustainability. Wild plants have played an important role in supplementing staple foods by supplying trace elements, vitamins and minerals in order to obtain a balanced diet, and they may do so again in the future. Their interest as a source of nutraceuticals has been highlighted in recent studies (Leonti, 2012). Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants reduces the risk of some cancers, heart and degenerative diseases. Nowadays, consumers appreciate substituting synthetic additives by natural additives, so the natural oxidants present in wild plants could be a possible alternative for agro food industries, as well as for gastronomy.

Moreover, some organic acids may have biological activity. Tartaric, malic, citric and succinic acids have shown health benefits as antioxidants due to their ability to chelate metals (Serrano et al., 2009). However, it is well known that oxalic acid has a negative effect on the reduction of the availability of dietary Ca and on the formation of kidney calculi (Guil et al., 1997). There is little published literature available about the comprehensive mineral and nutrient content of many wild plants. Such information is valuable not only for ascertaining the nutrient

contents that may be used to supplement the nutritional value of the normal diet, but also for environmentalists involved in the design of conservation programmes aimed at protecting and propagating nutritionally valuable wild plant resources. Moreover, some modern cultures still consume wild plants as a normal food source from which fairly high amounts of several nutrients are obtained, and it is widely accepted that leafy green vegetables are significant nutritional sources of minerals (Łuczaj et al., 2012; Rivera et al., 2006; Tardío et al., 2006).

At present, people in cities are accustomed to always consuming the same products cooked the same way, thus they are looking for new products in their meals. They need new colours, flavours, textures and ingredients (Mlcek & Rop, 2011); and therefore, many restaurant chefs are interested in finding and using plants that have been traditionally employed (Łuczaj et al., 2012). In this way, the study of many plant species that have been part of the Mediterranean diet is important, as they can be an interesting genetic resource for the development of new food products (Rivera et al., 2006; Tardío et al., 2006). Wild edible plants may have great potential as a source of unusual colours and flavours, bioactive compounds and as sources of dietary supplements or functional foods (Salvatore et al., 2005). In this regard, there is a great variety of wild non cultivated plants that have been employed in the diet and traditional medicine of the Mediterranean region (Pardo de Santayana et al., 2005), but their use has now decreased, and even disappeared, as a consequence of the industrial revolution.

In Spain and other European countries, wild edible plants have often been consumed, mostly as green vegetables. *Allium ampeloprasum*, *Asparagus acutifolius*, *Foeniculum vulgare*, *Malva sylvestris*, *Sanguisorba minor* and *Urtica urens* have been part of many traditional dishes, mainly in omelettes or

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scrambled eggs (Rivera et al., 2006; Tardío et al., 2006). Other species have been eaten raw as a snack: *Oxalis pes caprae* (peduncle of inflorescences) and *Malva sylvestris* (immature fruits) because of their acidic and sweet tastes, respectively. According to Hedrick (1972), the entire *Crithmum maritimum* plant is “of a spicy taste with a certain saltiness”, and has long been held in great repute as an ingredient in salads. It has been cultivated in English gardens and in France, but is more often collected from the shores. *Mesembryanthemum nodiflorum* and *M. cristalinum* have been consumed in some parts of the world. They are extremely tender, slightly salty and sour. Nowadays, some cooks at Spanish restaurants are using their leaves, cut into small pieces, as ingredients in salads. Acorns from *Quercus ballota* (there is a variety with sweet fruits) have been traditionally consumed in Spain; they are very nutritious due to their high carbohydrate content and can be used roasted and casserole, added to lentils or in cakes together with honey (Rivera et al., 2006). *Cistus albidus* petals have been eaten as a snack in the countryside and they have a nice pink colour that could be used to add colour to salads. Most of these plant species are good candidates as functional ingredients and could be included in the modern diet (Turner et al., 2011).

However, for these wild plants to become an alternative source for the food industry, broader knowledge about their physico-chemical, antioxidant and nutritional properties is necessary (Martins et al., 2011). These studies could be utilised by gastronomic companies to learn about the agro-industrial potential and promote their exploitation as additives or natural ingredients. It is generally acknowledged that studies on traditional knowledge systems should provide feedback to local communities and society in general (Leonti, 2012; Rivera et al., 2006).

The aim of this paper was to document the antioxidant properties, mineral composition, organic acids content and sensorial characteristics of different wild plant species traditionally used in Mediterranean areas and/or by different cooks in contemporary cuisine.

Materials and methods

Plant material

Different parts of 13 edible plants (Table 1) consumed in the Mediterranean diet were chosen according to previous ethno-botanical studies (Dogan, 2012; Łuczaj et al., 2013; Rivera et al., 2006; Tardío et al., 2006) and/or that they are presently used in European restaurants. These samples were gathered in southeast Spain from December 2009 to February 2010. Each species was collected at the moment they were suited for consumption according to their traditional use, and analyses were carried out on three samples of at least 100 g of the edible portions from 20 plants. Voucher specimens were deposited in the Miguel Hernandez University of Elche Herbarium (UMH). The assignment of species within families, the scientific nomenclature and the authority attribution for each taxon follow *The Plant List* (2012). The samples were lyophilised after collection, identified and kept in optimum conditions (-80°C and protected from light) for subsequent use. The experimental results were expressed as mean $\pm$ standard error (SE) of three replicates.

Antioxidant activity and total phenolics

Total antioxidant activity (TAA) was quantified according to Serrano et al. (2009), which enables the determination of TAA due to both hydrophilic and lipophilic compounds in the same extraction. The TAA (L TAA and H TAA) was determined using the enzymatic system composed by the chromophore 2,2'-azino bis (3 ethylbenzothiazoline 6 sulfonic acid) diammonium salt (ABTS), the horseradish peroxidase enzyme (HRP), and its oxidant

substrate (hydrogen peroxide, H_2O_2), in which $\text{ABTS}^{+\cdot}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the extract was proportional to the TAA in the sample. A calibration curve was performed with Trolox ((*R*)-(+)-6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) (0–20 μmol) from Sigma (Madrid, Spain), and the results are expressed as mg of Trolox equivalent 100 g^{-1} .

Total phenolic compounds were quantified according to the method described by Singleton et al. (1999) using the Folin Ciocalteu Reagent and the results are expressed as mg of gallic acid equivalent 100 g^{-1} .

Hydroxyl radical ($\text{OH}^{\cdot}$) and peroxy radical (H_2O_2) scavenging

Samples were prepared according to Egea et al. (2010). The lyophilised material (0.2 g) was extracted for 1 h by stirring at 4°C in darkness with 40 mL of phosphate buffer (10 mM KH_2PO_4 KOH buffer, pH 7.4), and centrifuged at $4500 \times g$ for 30 min. Aliquots of the supernatants were used as fruit extract in the different antioxidant assays.

Hydroxyl radical ($\text{OH}^{\cdot}$) scavenging potential assay: in a final volume of 1.2 mL, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4 KOH buffer (pH 7.4), 2.8 mM H_2O_2 , 2.8 mM deoxyribose (DR), 50 μM FeCl_3 pre-mixed with 100 μM EDTA before addition to the reaction mixture, and 100 μL of sample. Ascorbate (100 μM) was added to start the reaction. The tubes were incubated at 37°C for 1 h. The products of the hydroxyl radical ($\text{OH}^{\cdot}$) attack on sugar were measured as described by Egea et al. (2010) at 532 nm. The results are expressed as percentage inhibition of the DR attack, where 100% attack is defined as the absorbance levels recorded for DR without the addition of the samples. The same reaction without ASC was used to determine whether plants could reduce hydroxyl radical generation by avoiding the oxidation of ascorbate rather than by directly scavenging hydroxyl radicals.

Peroxy radical (H_2O_2) scavenging potential assay: an aliquot of the sample (100 μL) was incubated with 0.84 mM H_2O_2 for 10 min at 25°C . Aliquots of these mixtures were then taken and assayed for the remaining H_2O_2 by using the peroxidase system described by Murcia et al. (2004). The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 mL, 0.15 M KH_2PO_4 KOH buffer, pH 7.4, 0.5 mM guayacol, and 10 μL of Sigma type IV horseradish peroxidase. The results are expressed as percentage of disappearance of the H_2O_2 from the reaction medium.

Extraction and quantification of organic acids

The method described by Serrano et al. (2005), with some modifications, was employed. For the extraction, lyophilised material (0.1 g) was homogenated with 10 mL of deionised water at 50°C for 3 min. The final solution was then centrifuged at $24\,000 \times g$ for 30 min at room temperature. Subsequently, the extract was purified through Durapore 0.45 μm HV membrane filters (Millipore Corporation, Billerica, MA). The mobile phase was filtered through MFTM 0.45 μm HA membrane filters (Millipore Corporation). Organic acid quantification was carried out in a Hewlett Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1} . The organic acids were eluted through a Supelco column (Supelcogel C 610H, $30\text{ cm} \times 7.8\text{ mm}$, Supelco Park, Bellefonte, PA), and detected by absorbance at 210 nm. A standard curve of pure organic acids (oxalic, citric, malic and shikimic) purchased from Sigma (Poole, Dorset, UK) was used for quantification. Results are expressed as mg of acid 100 g^{-1} (percentage) fresh weight (FW).

Table 1. Plant species analysed.

Species	Family	Plant part analysed	English name	Traditional use
<i>Allium ampeloprasum</i> L.	<i>Amaryllidaceae</i>	Leaves	Great headed garlic. Levant Garlic. Wild leek	Salad
<i>Asparagus acutifolius</i> L.	<i>Asparagaceae</i>	Young shoots	Wild asparagus	Stew
<i>Cistus albidus</i> L.	<i>Cistaceae</i>	Petals	Rock rose	Snack, salad
<i>Crithmum maritimum</i> L.	<i>Apiaceae</i>	Leaves	Sea fennel, samphire, or rock samphire	Picked in vinegar
<i>Foeniculum vulgare</i> Mill.	<i>Apiaceae</i>	Leaves, young shoots	Fennel	Snack, salad, stew
<i>Malva sylvestris</i> L.	<i>Malvaceae</i>	Leaves	High mallow, Marsh mallow	Stew
<i>Malva sylvestris</i> L.	<i>Malvaceae</i>	Immature fruits	Cheeses	Snack, salad
<i>Mesembryanthemum nodiflorum</i> L.	<i>Aizoaceae</i>	Leaves	Slender leaf ice plant	Salad
<i>Mesembryanthemum cristalinum</i> L.	<i>Aizoaceae</i>	Leaves	Crystalline ice plant, ice plant	Salad
<i>Oxalis pes caprae</i> L.	<i>Oxalidaceae</i>	Leaves and peduncle of inflorescence	Cape sorrel	Salad
<i>Quercus ballota</i> Desf.	<i>Fagaceae</i>	Fruits (acorns)	Ballota, beloot or belote oak. Holly oak	Raw, boiled, roasted
<i>Sanguisorba minor</i> Scop.	<i>Rosaceae</i>	Leaves	Burnet	Salad
<i>Sedum sediforme</i> Pau	<i>Crassulaceae</i>	Leaves	Pale stonecrop, white stonecrop	Picked in vinegar
<i>Urtica urens</i> L.	<i>Urticaceae</i>	Tender leaves and shoots	Nettle	Stew

Nutrient minerals

Na and K contents were determined in nitric acid (HNO₃): perchloric acid (HClO₄) (2:1 v/v) digestion extract by Jenway PFP7 flame photometry (Bibby Scientific, Dunmow, UK), and Ca and Mg, Fe and Zn by Unicam Solaar 969 atomic absorption spectrophotometry (Unicam, Cambridge, UK).

Sensory attributes

Ten trained tasters judged five sensory attributes in a quantitative manner: sweetness, acidity, bitterness, aroma and juiciness. To value each attribute, the scale ranged from 0 (minimum value) to 10 (maximum value). The tasters valued the five sensory attributes on raw samples from six species that traditionally have been consumed in salad: *Cistus albidus*, *Malva sylvestris* (immature fruits), *Mesembryanthemum nodiflorum*, *M. cristalinum*, *Oxalis pes caprae* and *Sanguisorba minor*. The other species (*Allium ampeloprasum*, *Asparagus acutifolius*, *Foeniculum vulgare*, *Malva sylvestris* (leaves) *Quercus ballota*, *Urtica urens*, *Sedum sediforme* and *Crithmum maritimum*) traditionally have been consumed after boiling, being part of casseroles or pickled in vinegar, therefore they were evaluated after boiling with tap water in a 1:5 w/v proportion for 15 min. The tasters also made a global quantitative assessment of each species and qualitatively highlighted the most relevant properties.

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA). The results were calculated using statistical software (SPSS 14.0, SPSS Inc., Chicago, IL). Correlations between different parameters were computed as Pearson's correlation coefficient (*r*). Least significant difference values (LSD, $p \leq 0.05$) were used to compare the means.

Results and discussion

Antioxidant activity (hydrophilic and lipophilic antioxidant activities) and total phenolic content

The highest H TAA (Figure 1a) was found in *Sanguisorba minor*, *Quercus ballota* and *Sedum sediforme*, with 1368.91 $\pm$ 47.96, 1186.91 $\pm$ 109.31 and 588.87 $\pm$ 35.52 mg of Trolox equivalent 100 g⁻¹ FW, respectively. Such high levels of H TAA are found in

some fruits or parts of plants employed for medicinal use (Surveswaran et al., 2007) and in other non cultivated fruits like blackberry, blueberry or strawberry trees. This extremely high antioxidant capacity has been attributed to their high phytonutrient content (García Alonso et al., 2004). Other species studied, like *Cistus albidus* (petals) and *Malva sylvestris* (leaves), also showed a high antioxidant capacity, with values of 132.14 $\pm$ 4.91 and 77.92 $\pm$ 5.71 mg of Trolox equivalent 100 g⁻¹ FW, respectively, although their activity was about 20 times lower than *S. minor* and *Q. ballota* fruits. Dall'Acqua et al. (2008) and Egea et al. (2010) reported that *S. minor* could be considered a possible new antioxidant ingredient for the nutraceutical or functional food market. The remaining analysed species and immature fruits from *M. sylvestris* showed antioxidant capacity that was about 50 times lower than *S. minor* and *Q. ballota*. Although these activities are not very high, they are similar to grapefruits and oranges, and higher than the antioxidant activity of other fruits like tomato, banana, peach or apple (Proteggente et al., 2002).

L TAA (Figure 1b) followed a pattern similar to H TAA (Figure 1a), thus plants with the highest L TAA were again *Q. ballota*, *S. sediforme* and *S. minor*. However, in the first two species, the L TAA was 40% and 45% of the total antioxidant activity, while in *S. minor* it was only 10% of the total. No literature is available to contrast the L TAA results in plants; however, the presence of tocopherol has been related to L TAA in some fruits (Cho et al., 2007). The high L TAA has also been related to high levels of carotenoids in sweet cherry cultivars (Serrano et al., 2009).

The main antioxidant substances found in fruits and plants include ascorbic acid, carotenoids and phenolic compounds (Egea et al., 2010; Murcia et al., 2004). The plant with the highest total phenol levels (530.51 $\pm$ 21.12 mg 100 g⁻¹ FW) was *S. minor* (Figure 1c), whose levels were similar to those found in several cultivars of *Fragaria vesca*, the fruit which is considered to have elevated levels of phenolic compounds (Doumet et al., 2011). Consistent with our results, Vanzani et al. (2011) found particularly high levels of polyphenols and ferric reducing antioxidant power in *S. minor*. The *Q. ballota*, *C. albidus* and *S. sediforme* species also showed high levels of phenolic compounds, 326.91 $\pm$ 51.05, 269.46 $\pm$ 21.15 and 191.53 $\pm$ 13.78 mg 100 g⁻¹ FW, respectively. These values are similar and even higher to those found by Proteggente et al. (2002) in regularly consumed fruits considered to have a high polyphenol content, such as strawberry (330 mg 100 g⁻¹ FW), red plum (320 mg 100 g⁻¹ FW)

Figure 1. (a) H TAA, hydrophilic antioxidant activity (mg eq. Trolox 100 g^{-1} FW), (b) L TAA, lipophilic antioxidant activity (mg eq. Trolox 100 g^{-1} FW) and (c) total phenolic content (mg 100 g^{-1} FW) of different wild edible plants. Data are the mean $\pm$ SE. LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

or raspberry ($228\text{ mg } 100\text{ g}^{-1}$ FW). Most species in this study presented between 20 and $80\text{ mg } 100\text{ g}^{-1}$ FW of phenolic compounds; nevertheless, they can also be considered as a good natural source of polyphenols. These levels are similar to those found in other fruits like deerberry (Marinova et al., 2005), orange, red cabbage and broccoli (Proteggente et al., 2002). Finally, the species with the lowest phenol levels was *Oxalis pes*

caprae with $11.56 \pm 0.21\text{ mg } 100\text{ g}^{-1}$ FW, below the level found in most regularly consumed fruits (Proteggente et al., 2002).

In this study, a positive relationship was found between H TAA and total phenolics in most of the species considered according to the equation $y = 2.7x - 83$ with $r^2 = 0.81$. Although not determined in this study, it is well known that ascorbic acid can also contribute to H TAA (Serrano et al., 2005). However, the activity

in the H TAA assay increases with the number of hydroxyl groups in the antioxidant compound, so antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts under experimental conditions. This relation between total phenols and antioxidant activity has been found in many fruits (Proteggente et al., 2002; Serrano et al., 2009), and suggests that the phenolic compounds could also be mostly responsible for the antioxidant properties of *S. minor* and *Q. ballota*. Recently, it has been reported that the ingestion of certain foods containing high amounts of phenolic compounds has an antioxidant activity *in vivo* by increasing plasmatic antioxidants (Fernández Panchón et al., 2008).

Hydroxyl radical (OH•) and peroxy radical (H₂O₂) scavenging

Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are capable of damaging almost every molecule found in living cells. These radicals have the

percentages of oxidative attack lower than 35%, similar to cinnamon and vanilla (Murcia et al., 2004), and thus, they could be considered with a low hydroxyl radical (OH•) scavenging potential. Their inhibition percentages are near the synthetic antioxidant BHT and propyl gallate (Egea et al., 2010); therefore, they could be considered a prooxidant in the deoxyribose assay and probably can have a synergistic effect with ASC, increasing the concentration of hydroxyl radicals in the medium and consequently stimulating sugar degradation (Murcia et al., 2001).

There is increasing evidence that H₂O₂, either directly or indirectly via its reduction product OH•, may act as a messenger molecule in the synthesis and activation of several inflammatory mediators. The ability of different extracts of edible plants to scavenge H₂O₂ is shown in Table 2. *Mesembryanthemum nodiflorum*, *S. sediforme* and *U. urens* exhibited the highest activity, similar to some wild edible fruits like *Rosa canina* and *Crataegus monogyna* (Egea et al., 2010). However, *M. sylvestris* (immature fruits) and *O. pes caprae* had a very low capacity for scavenging H₂O₂ (10.05% and 4.09% of inhibition, respectively),

Table 2. Scavenging radical OH• capacity, and H₂O₂ scavenging of different wild edible plants (% Inhibition) (mean ± SE).

Species	Oxidative attack to DR (% Inhibition) ^a With ASC	Oxidative attack to DR (% Inhibition) ^a Without ASC	Scavenging H ₂ O ₂ (% Inhibition) ^a
<i>Allium ampeloprasum</i> L.	50.46 ± 0.36	29.84 ± 0.68	22.21 ± 6.67
<i>Asparagus acutifolius</i> L.	53.49 ± 1.93	59.79 ± 4.34	62.45 ± 3.42
<i>Cistus albidus</i> L.	31.18 ± 2.56	60.19 ± 1.15	47.48 ± 4.39
<i>Crithmum maritimum</i> L.	52.88 ± 1.71	68.99 ± 11.12	65.30 ± 6.71
<i>Foeniculum vulgare</i> Mill.	46.51 ± 4.69	61.75 ± 7.53	63.55 ± 4.46
<i>Malva sylvestris</i> L. (leaves)	47.16 ± 8.01	81.33 ± 1.67	35.10 ± 6.12
<i>Malva sylvestris</i> L. (immature fruits)	9.90 ± 1.32	65.62 ± 3.52	10.05 ± 3.54
<i>M. nodiflorum</i> L.	42.02 ± 6.50	65.71 ± 1.14	80.49 ± 0.82
<i>M. cristalinum</i> L.	1.38 ± 0.63	70.38 ± 1.08	74.88 ± 2.03
<i>Oxalis pes caprae</i> L.	30.25 ± 6.34	83.81 ± 4.86	4.09 ± 1.99
<i>Quercus ballota</i> Desf.	31.07 ± 4.41	20.64 ± 3.63	29.90 ± 4.37
<i>Sanguisorba minor</i> Scop.	57.75 ± 9.30	33.50 ± 3.09	64.35 ± 5.52
<i>Sedum sediforme</i> Pau.	43.60 ± 3.61	12.48 ± 3.79	81.60 ± 4.97
<i>Urtica urens</i> L.	57.09 ± 2.99	77.85 ± 2.42	81.41 ± 0.36
LSD _{0.05} ^b	6.44	9.73	6.03

DR Deoxyribose; ASC Ascorbate.

^aPercentage of inhibition due to the extracts at the final concentration of 5 mg/ml.

^bLSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

capacity to join the nucleotides in DNA and cause strand breakage, contributing to carcinogenesis, mutagenesis and cytotoxicity. In addition, this species is considered as one of the quick initiators of the lipid peroxidation process due to abstraction of hydrogen atoms from unsaturated fatty acids (Murcia et al., 2004). Table 2 shows the inhibition of hydroxyl radicals by extracts of different edible plants. Based on their antioxidant capacity, the species considered in this work can be divided into three groups. In the first are *Allium ampeloprasum*, *Asparagus acutifolius*, *Crithmum maritimum*, *Foeniculum vulgare*, *M. sylvestris* (leaves), *Mesembryanthemum nodiflorum*, *S. minor*, *S. sediforme* and *Urtica urens*, with inhibition percentages between 40% and 60%. Therefore, according to this assay, they can be considered moderately powerful antioxidants. They have a similar inhibition percentage such as other spices like anise, ginger, licorice, mint and nutmeg (Murcia et al., 2004) and different edible wild fruits (*Rubus ulmifolius* and *Sorbus domestica*) (Egea et al., 2010). From this group, *S. minor* and *U. urens* are the species presenting a higher protector effect of deoxyribose (DR) against oxidative attack, with inhibition percentages of $57.75 \pm 9.3\%$ and $57.09 \pm 2.99\%$, respectively. The second group of plants, with *C. albidus*, *O. pes caprae* and *Q. ballota*, showed inhibition

similar, in this case, to other wild edible fruits like *Crataegus azarolus* and *S. domestica* (Egea et al., 2010). In this assay, N acetyl L cysteine, with a 97.04% hydrogen peroxide scavenging ability, was used as positive hydrogen peroxide scavenger control.

Considering the activity obtained in the different antioxidant assays, no relationship was found between TAA and the ability to capture the OH• and H₂O₂ radicals. The differences shown by the same plants in different antioxidant tests may be due to the different reaction media and the different chemical natures of the radical species generated (Murcia et al., 2004). The different antioxidant activity of the 14 extracts from the wild edible plants may be due to their different antioxidant composition.

Organic acids

Different organic acids were identified and quantified: oxalic, citric, malic and shikimic (Figure 2). Oxalic acid is abundant in many leafy vegetables and, in general, a very wide variability in this organic acid content has been found in the literature (Guil et al., 1997; Sánchez Mata et al., 2012). In this study, very high levels of oxalic acid were found in *O. pes caprae* (1148 ± 67.9 mg 100 g⁻¹ FW) and in both *Mesembryanthemum* species, around

Figure 2. Oxalic acid (Ox), citric acid (Ct), malic acid (MI) and shikimic acid (Sk) ($\text{mg } 100 \text{ g}^{-1} \text{ FW}$) of different wild edible plants. Data are the mean $\pm$ SE. Inner figure: HPLC profile of oxalic acid in *M. cristalinum*. Data are the mean $\pm$ SE. $\text{LSD}_{0.05}$ Ox 26.43; Ct 56.36; MI 63.65; Sk 2.12. LSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

$900 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$. These levels are significantly greater than those found in the other species of this study ($p < 0.05$). The amount of oxalic acid is higher than that of *Beta maritima* (around $500 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$), considered by Guil et al. (1997) and Sánchez Mata et al. (2012) as a species with a high oxalic acid content. In the present study, low oxalic acid levels were found in other species, like *S. minor*, with $103.28 \pm 5.18 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, and the species presenting less than $30 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$ were *F. vulgare*, *U. urens*, *A. acutifolius* and *C. maritimum*. Sánchez Mata et al. (2012) reported oxalic acid levels of 123.82 and $402.83 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$ in wild *F. vulgare* from different origins, and Souci et al. (2008) indicated a level of $5 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$ in cultivated *F. vulgare*. Oxalic acid has quite a low toxicity, with 5 g as the minimal lethal dose for an adult. However, the presence of oxalates in plants can interfere with the absorption of Ca and contribute to the formation of oxalate kidney calculi (Guil et al., 1997). For this reason, ingesting high levels of this organic acid is not desirable, although *O. pes caprae* and both *Mesembryanthemum* species could be consumed in small amounts in salads.

In most plant species, the main acids are citric and malic (Oliveira et al., 2008; Sanchez Mata et al., 2012). In the present work and of all the species studied, *S. sedifforme* presented the highest concentration of citric and malic acids, 1717 ± 8.97 and $1508 \pm 161 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, respectively ($p < 0.05$). Other species with remarkable amounts of citric acid were *M. cristalinum*, *M. sylvestris* (leaves) and *U. urens* with 408 ± 15 , 561.91 ± 26 and $458.1 \pm 9 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, respectively. *A. acutifolius*, with $362.5 \pm 25.7 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, had similar levels of citric acid to those found by Souci et al. (2008) in *Asparagus officinalis* ($350 \text{ mg } 100 \text{ g}^{-1}$). An indirect contribution of citric acid to the entire antiradical activity of some berries could be related to its inhibition properties towards phenolase and polyphenol oxidase enzymes, which are very important for regulating the concentration of polyphenols (Jiang et al., 2004). Therefore, it is possible that citric and malic acids indirectly contribute to the high antioxidant capacity of some of the considered species like *S. sedifforme* (Figure 1). In relation to shikimic acid, the quantities

found in *S. sedifforme* and *C. albidus* were 135.3 ± 3.3 and $100.3 \pm 4.8 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$. This organic acid is important as most of the phenolic compounds derived from the shikimic acid pathway.

Mineral composition

Mineral elements were found to vary widely depending on the species. Results from the mineral analysis are given in $\text{mg } 100 \text{ g}^{-1}$ of dry weight (Table 3). As expected, the Na content was found to be greater in halophytic vegetables, ranging from $50 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ in *Allium ampeloprasum* to 12513 and $13231 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ in *M. cristalinum* and *M. nodiflorum*, respectively. These last values indicate that those species should be consumed without salt addition. The potassium concentration was high in most cases. The lowest value, $980 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, was found in *Q. ballota*, and there were values above $4000 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ in *A. acutifolius*, *F. vulgare*, leaves of *M. sylvestris*, *M. cristalinum* and *O. pes caprae*. That K concentration resulted much higher than in banana ($1500 \text{ mg K } 100 \text{ g}^{-1} \text{ DW}$, USDA ARS, 1998), a fruit considered to have a high K content.

Calcium and Mg nutrition in humans are important concerns due to the critical role they play in bone development and structure, and in cellular metabolism. Dairy products supply a majority of human dietary Ca and Mg requirements; however, certain vegetables can also provide significant amounts of these minerals (Farnham et al., 2000). Spinach has a high Ca concentration, but this occurs in association with oxalates that bind Ca and decrease the mineral's bioavailability. Other vegetables, like broccoli, have relatively high Ca concentrations and low levels of the constituent that renders Ca unavailable. Broccoli has been cited in the popular press as one of the best vegetable sources of Ca in the human diet. Farnham et al. (2000) indicated an average value of $340 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ of Ca in different varieties of broccoli. Considering the species in the present work, the lowest Ca content was found in *Allium* and *Asparagus* (373 and $367 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, respectively); medium values ranged from 2858 to $3355 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ in *M. cristalinum*,

Table 3. Mineral content of different parts of different wild edible plants (mg 100 g⁻¹ DW) (mean ± SE).

Species	Na	K	Ca	Mg	Fe	Zn
<i>Allium ampeloprasum</i> L.	50.0 ± 5.1	2767 ± 291	373 ± 35	93 ± 6	2.22 ± 0.05	3.48 ± 0.05
<i>Asparagus acutifolius</i> L.	112.8 ± 5.1	4167 ± 120	367 ± 12	157 ± 7	6.08 ± 0.20	13.82 ± 0.67
<i>Cistus albidus</i> L.	103.6 ± 12.6	1200 ± 0	650 ± 44	133 ± 6	7.15 ± 0.14	5.02 ± 0.34
<i>Crithmum maritimum</i> L.	4820 ± 135	1500 ± 115	3137 ± 21	435 ± 28	8.29 ± 0.85	6.59 ± 0.84
<i>Foeniculum vulgare</i> Mill.	427 ± 36	4567 ± 20	3355 ± 108	318 ± 12	8.09 ± 0.68	8.35 ± 1.6
<i>Malva sylvestris</i> L. (leaves)	538 ± 18	4067 ± 120	890 ± 0	110 ± 0	22.48 ± 2.64	13.5 ± 0.62
<i>Malva sylvestris</i> L. (immature fruits)	251 ± 5	2033 ± 33	1280 ± 28	333 ± 2	8.81 ± 2.32	8.06 ± 0.41
<i>M. nodiflorum</i> L.	13 231 ± 514	3100 ± 250	2995 ± 53	675 ± 31	7.45 ± 3.29	3.24 ± 0.95
<i>M. cristalinum</i> L.	12 513 ± 420	4700 ± 200	2858 ± 107	602 ± 26	13.49 ± 1.84	9.01 ± 0.41
<i>Oxalis pes caprae</i> L.	297 ± 5	4033 ± 240	782 ± 34	145.0 ± 8.7	8.47 ± 3.28	3.67 ± 0.49
<i>Quercus ballota</i> Desf.	32.3 ± 1.4	980 ± 22	81 ± 9	78.3 ± 3.5	1.48 ± 0.1	3.01 ± 0.08
<i>Sanguisorba minor</i> Scop.	67.2 ± 2.5	1850 ± 95	1772 ± 70	586 ± 52	21.20 ± 4.02	4.81 ± 0.17
<i>Sedum sediforme</i> Pau	90.8 ± 16.2	1467 ± 186	8112 ± 641	260 ± 29	7.12 ± 0.70	8.20 ± 0.7
<i>Urtica urens</i> L.	169.2 ± 8.9	3000 ± 58	9275 ± 155	965 ± 8	9.38 ± 0.70	7.99 ± 1.13
LSD _{0.05} ^a	247.49	211.17	250.22	26.81	2.72	1.01

^aLSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

Table 4. Global assessment by the consumer and most relevant properties for modern diets of different wild edible plants (mean ± SE).

Species	Mode of consumption by the tester panel ^a	Sweetness ^b	Acidity ^b	Bitterness ^b	Aroma ^b	Juiciness ^b	Global acceptance ^b	Most relevant properties for modern diets ^c
<i>Allium ampeloprasum</i> L.	Boiled	6.88 ± 0.75	3.13 ± 0.13	2.75 ± 0.69	8.63 ± 0.53	6.88 ± 0.42	8.13 ± 0.33	Aroma, spicy taste
<i>Asparagus acutifolius</i> L.	Boiled	7.67 ± 0.17	2.00 ± 0.58	5.67 ± 0.17	8.00 ± 0.29	7.67 ± 0.44	9.67 ± 0.17	Aroma, texture
<i>Cistus albidus</i> L.	Raw	6.40 ± 0.52	1.20 ± 0.38	2.00 ± 0.58	2.40 ± 0.33	4.60 ± 0.70	5.80 ± 0.38	Colour, appearance
<i>Crithmum maritimum</i> L.	Boiled	4.33 ± 1.01	7.00 ± 0.58	5.33 ± 1.36	9.00 ± 0.29	7.67 ± 0.73	2.01 ± 0.50	Texture, salty taste
<i>Foeniculum vulgare</i> Mill.	Boiled	6.38 ± 0.84	3.13 ± 0.57	3.25 ± 1.02	9.00 ± 0.46	4.00 ± 0.87	5.13 ± 0.78	Aroma
<i>Malva sylvestris</i> L. (leaves)	Boiled	5.20 ± 0.52	1.80 ± 0.47	2.40 ± 0.72	4.00 ± 0.54	5.80 ± 0.63	5.01 ± 0.46	Texture, appearance
<i>Malva sylvestris</i> L. (immature fruits)	Raw	5.20 ± 0.59	4.20 ± 0.99	4.40 ± 0.95	3.20 ± 0.80	5.20 ± 1.03	5.20 ± 0.75	Texture
<i>M. nodiflorum</i> L.	Raw	2.25 ± 0.72	2.00 ± 0.78	1.50 ± 0.29	4.25 ± 0.60	6.50 ± 0.76	5.02 ± 0.24	Texture, salty taste
<i>M. cristalinum</i> L.	Raw	2.00 ± 0.58	3.00 ± 0.85	1.25 ± 0.55	5.25 ± 0.49	8.00 ± 0.24	6.00 ± 0.24	Texture, salty taste
<i>Oxalis pes caprae</i> L.	Raw	1.75 ± 0.28	8.75 ± 0.14	4.00 ± 1.08	3.75 ± 0.49	5.50 ± 0.76	4.00 ± 0.62	Texture, acid taste
<i>Quercus ballota</i> Desf.	Boiled	8.50 ± 0.37	0.75 ± 0.14	2.75 ± 0.28	9.50 ± 0.17	2.75 ± 0.28	9.25 ± 0.28	Aroma, sweet taste
<i>Sanguisorba minor</i> Scop.	Raw	2.60 ± 0.44	3.00 ± 0.54	4.40 ± 1.01	2.60 ± 0.44	3.40 ± 0.39	4.8 ± 0.12	Appearance
<i>Sedum sediforme</i> Pau	Boiled	2.00 ± 0.29	4.67 ± 0.17	5.33 ± 0.17	7.33 ± 0.17	1.67 ± 0.17	1.67 ± 0.17	Texture, appearance
<i>Urtica urens</i> L.	Boiled	5.25 ± 0.86	4.00 ± 0.75	3.75 ± 0.76	6.25 ± 0.60	6.50 ± 0.69	5.00 ± 0.47	Texture
LSD _{0.05} ^d		0.84	0.78	1.06	0.65	0.86	0.61	

^aMode of consumption by the tester panel (raw or boiled).

^bSweetness, acidity, bitterness, aroma, juiciness, global acceptance (from 0, minimum value of sweetness to 10, the maximum value).

^cMost relevant properties for creative cuisine were determined by the tester panel.

^dLSD ($p < 0.05$), least significant difference, was used for significant difference comparison among means of various samples.

M. nodiflorum, *C. maritimum* and *F. vulgare*, and the highest content was found in *S. sediforme* and *U. urens* (8112 and 9275 mg 100 g⁻¹ DW, respectively). Therefore, these species present a much higher Ca concentration than broccoli, and the consumption of 100 g FW of the last two species would satisfy daily Ca requirements (800 mg). The impact of inadequate Mg is not as significant or as well recognized as it is for Ca, and it is considered that no Mg deficiencies are found in normal diets. Magnesium contents varied from 78 mg 100 g⁻¹ DW (*Q. ballota*) to 965 mg 100 g⁻¹ PS in *U. urens*.

The lowest iron concentration was found in *Q. ballota* (1.48 mg) and in *A. ampeloprasum* (2.2 mg), while the highest occurred in *S. minor* (21.2 mg) and *M. sylvestris* leaves (22.48 mg). The Fe content in raw spinach is 2.71 mg 100 g⁻¹ FW (USDA ARS, 1998), thus around 27 mg 100 g⁻¹ DW, which indicates that *S. minor* and *M. sylvestris* leaves could be good sources of Fe, like spinach. An intake of around 15 mg per day of Zn, a trace element that is especially important for the normal function of the immune system, is required. Zinc was relatively abundant in *Asparagus* (13.82 mg 100 g⁻¹) and in leaves of *M. sylvestris* (13.5 mg

100 g⁻¹), while its level in *Q. ballota* (3.01 mg 100 g⁻¹) was lower than in the remaining the species.

Sensory properties

From the consumer's point of view (Table 4), the following species presented a medium sweetness value (between 6 and 8): *A. ampeloprasum*, *A. acutifolius*, *C. albidus* and *F. vulgare*, while fruits from *Q. ballota* could be considered very sweet (8.5 ± 0.37). These species could be used as ingredients providing sweetness in salads or other meals. On the other hand, in relation to acidity, *C. maritimum* and *O. pes caprae* showed the maximum levels, with 7 ± 0.58 and 8.75 ± 0.14, respectively. Most species from this study were not especially bitter.

The most aromatic plants were *A. ampeloprasum*, *A. acutifolius*, *C. maritimum*, *F. vulgare* and *Q. ballota*, with values higher than 8. *A. ampeloprasum*, *A. acutifolius*, *C. maritimum*, *M. nodiflorum*, *M. cristalinum*, *S. sediforme* and *U. urens* were considered especially juicy, with values between 6.5 and 8. Moreover, the panel of tasters highlighted some characteristics from the plants that make them suitable for use as

supplements to improve taste, aroma or texture. Three species stood out for their salty taste: *C. maritimum*, *M. nodiflorum* and *M. cristalinum*, while *A. ampeloprasum* could add a slightly spicy taste to meals, and *O. pes caprae* and *Q. ballota* could contribute with very acidic or sweet tastes, respectively.

Petals from *C. albidus* were highlighted for their pink colour and *F. vulgare* for its aroma, while the rest of the species (i.e. *S. sedifforme*, *M. sylvestris*, *U. urens*) were appreciated for their appearance, different from the remaining commercial vegetables. These plants, like *S. minor*, could be used in salads. However, when the global appreciation was evaluated, only four species obtained a positive value (between 6 and 10): *A. ampeloprasum*, *A. acutifolius*, *M. cristalinum* and *Q. ballota*. But it is noteworthy that these values (Table 4) are the results from the evaluation of each individual parameter using raw or boiled material. These plants used with condiments, or dressed in salads, may be appreciated more by the consumer.

Conclusions

In summary, taking into account both total antioxidant activity and the phenolic content of the plants analysed, *S. minor*, *Q. ballota*, *S. sedifforme* and *C. albidus* could be used as nutritional supplements in functional foods with high antioxidant activity and as natural antioxidants to replace synthetic ones. In addition, *S. sedifforme* also provides a high amount of calcium to the diet, while *S. minor* and the pink flowers of *C. albidus* would provide colour and enhance the appearance of salads. Both *Mesembryanthemum* species and *O. pes caprae* would only be recommended in small quantities due to their high oxalic acid content, although they would provide pleasant salty and acidic tastes, respectively. Moreover, these wild plants have a greater mineral content than some cultivated vegetables. Thus, the consumption of the investigated plant species could provide health benefits due to their high antioxidant and nutritional values as well as their sensorial properties. These plants could be used to improve ingredient diversity in modern cuisine.

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Declaration of interest

The authors declare that they have no conflict of interest.

References

- Cho YS, Yeum KJ, Chen CY, Beretta G, Tang G, Krinsky N, Yoon S, et al. 2007. Phytonutrients affecting hydrophilic and lipophilic antioxidant activities in fruits, vegetables and legumes. *J Sci Food Agriculture* 87:1096–1107.
- Dall'Acqua S, Cervellati R, Loi MC, Innocenti G. 2008. Evaluation of in vitro antioxidant properties of some traditional Sardinian medicinal plants: investigation of the high antioxidant capacity of *Rubus ulmifolius*. *Food Chem* 106:745–749.
- Dogan Y. 2012. Traditionally used wild edible greens in the Aegean Region of Turkey. *Acta Soc Botanic Polo* 81:329–342.
- Doumett S, Fibbi D, Cincinelli A, Giordani E, Nin S, Del Bubb, M. 2011. Comparison of nutritional and nutraceutical properties in cultivated fruits of *Fragaria vesca* L. produced in Italy. *Food Research International* 44:1209–1216.
- Egea I, Sánchez Bel P, Romojaro F, Pretel MT. 2010. Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods Human Nutr* 65:121–129.
- Farnham MW, Grusak MA, Wang M. 2000. Calcium and magnesium concentration of inbred and hybrid broccoli heads. *J Am Soc Horticultural Sci* 125:344–349.
- Fernández Panchón MS, Villano D, Troncoso AM, García Parrilla MC. 2008. Antioxidant activity of phenolic compounds: from in vitro results to in vivo evidence. *Crit Rev Food Sci Nutr* 48:649–667.
- García Alonso M, De Pascual TS, Santos Buelga C, Rivas Gonzalo JC. 2004. Evaluation of the antioxidant properties of fruit. *Food Chem* 84:13–18.
- Guil JL, Rodríguez I, Torija ME. 1997. Nutritional and toxic factors in selected wild edible plants. *Plant Foods Human Nutr* 51:99–107.
- Hedrick UP. 1972. *Sturtevant's edible plants of the world*. New York: Dover Publications.
- Jiang Y, Penb L, Lia J. 2004. Use of citric acid for shelf life and quality maintenance of fresh cut Chinese water chestnut. *J Food Eng* 63:325–328.
- Leonti M. 2012. The co evolutionary perspective of the food medicine continuum and wild gathered and cultivated vegetables. *Gen Res Crop Evol* 59:1295–1302.
- Łuczaj Ł, Marijana ZK, Tihomir M, Katija D, Marija P. 2013. Wild vegetable mixes sold in the markets of Dalmatia (southern Croatia). *J Ethnobiol Ethnomed* 9:2. doi:10.1186/1746-4269-9-2.
- Łuczaj Ł, Pieroni A, Tardío J, Pardo de Santayana M, Sõukand R, Svanberg I, Kalle R. 2012. Wild food plant use in 21st century Europe: the disappearance of old traditions and the search for new cuisines involving wild edibles. *Acta Soc Botan Polo* 81:359–370.
- Marinova D, Ribarova F, Atanassova M. 2005. Total phenolics and total flavonoids in Bulgaria fruits and vegetables. *J Univ Chem Technol Metal* 40:255–260.
- Martins D, Barros L, Carvalho AM, Ferreira IC. 2011. Nutritional and in vitro antioxidant properties of edible wild greens in Iberian Peninsula traditional diet. *Food Chem* 125:488–494.
- Mrcek J, Rop O. 2011. Fresh edible flowers of ornamental plants – a new source of nutraceutical foods. *Trends Food Sci Technol* 22:561–569.
- Murcia MA, Egea I, Romojaro F, Parras P, Jimenez AM, Martínez Tome M. 2004. Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. *J Agric Food Chem* 52:1872–1881.
- Murcia MA, Jimenez AM, Martinez Tome M. 2001. Evaluation of the antioxidant properties of Mediterranean and tropical fruits compared with common food additives. *J Food Protect* 64:2037–2046.
- Oliveira AP, Pereira JA, Andrade PB, Valentão P, Seabra RM, Silva BM. 2008. Organic acids composition of *Cydonia oblonga* Miller leaf. *Food Chem* 111:393–399.
- Pardo de Santayana M, Tardío J, Morales R. 2005. The gathering and consumption of wild edible plants in the Campoo (Cantabria, Spain). *Int J Food Sci Nutr* 56:529–542.
- Proteggente AR, Pannala AS, Paganga G, Van Buren L, Wagner E, Wiseman S. 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Rad Res* 36:217–233.
- Rivera D, Obón C, Heinrich M, Inocencio C, Verde A, Fajardo J. 2006. Gathered Mediterranean food plants – ethnobotanical investigations and historical development. In: Heinrich M, Müller WE, Galli C, editors. *Local Mediterranean food plants and nutraceuticals*. Basel, Karger: Forum Nutrition. p 18–74.
- Salvatore S, Pellegrini N, Brenna OV, Del Rio D, Frasca G, Brighenti F, Tumino R. 2005. Antioxidant characterization of some Sicilian edible wild greens. *J Agric Food Chem* 53:9465–9471.
- Sánchez Mata MC, Cabrera Loera RD, Morales P, Fernández Ruiz V, Cámara M, Díez Marqués C, Pardo de Santayana M, Tardío J. 2012. Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds. *Gen Res Crop Evol* 59:431–443.
- Serrano M, Díaz Mula HM, Zapata PJ, Castillo S, Guillén F, Martínez Romero D, Valverde JM, 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, Guillén F, Martínez 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 VL, Orthofer R, Lamuela Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Meth Enzymol* 299:152–178.
- Souci SW, Fachmann W, Kraut H. 2008. *Food composition and nutrition tables*. (7th revised and completed edition). Stuttgart: Medpharm Scientific Publishers.
- Surveswaran S, Cai YZ, Corke H, Sun M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chem* 102:938–953.

- Tardío J, Pardo de Santayana M, Morales R. 2006. Ethnobotanical review of wild edible plants in Spain. *Botan J Linnean Soc* 152:27–71.
- The Plant List. 2012. A working list of all plant species. Available at: <http://www.theplantlist.org/>. Accessed on 10 July 2012.
- Turner NJ, Łuczaj ŁJ, Migliorini P, Pieroni A, Dreon, AL, Sacchetti, LE, Paoletti MG. 2011. Edible and tended wild plants, traditional ecological knowledge and agroecology. *Crit Rev Plant Sci* 30:198–225.
- US Department of Agriculture Agricultural Research Service (USDA ARS). 1998. USDA nutrient database for standard reference, release 12. Nutrient Data Laboratory Home Page. Available at: <http://www.nal.usda.gov/fnic/foodcomp>. Accessed on 05 July 2012.
- Vanzani P, Rossetto M, De Marco V, Sacchetti LE, Paoletti MG, Rigo A. 2011. Wild Mediterranean plants as traditional food: a valuable source of antioxidants. *J Food Sci* 76:46–51.

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Title: Wild edible plants as potential antioxidant or nutritional supplements for beverages minimally processed

Article Type: Research Article

Keywords: wild edible plants, nutritional supplement, antioxidant potential, fruit, minimal processing

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Abstract: Wild edible plants (WEP) with high antioxidant capacity, *Rosa canina* fruits, *Quercus ballota* acorns and *Sanguisorba minor* (young leaves and stems), were added to orange and kiwifruit juices for increase the nutritional properties. The addition of WEP to orange juice (OJ) and kiwifruit cremogenate (KC) does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued. The beverages minimally processed with higher TSS were the matrices fortified with *Q. ballota*, the same that were considered sweetest by the judges. The addition of WEP to OJ did not significantly change the levels of vitamin C (ascorbic acid plus dehydroascorbic acid). The addition of *R. canina* and *S. minor* increased the content of phenolic compounds and antioxidant capacity in both matrices (OJ and KC) in more than 30 %. Beverages with a high interest for consumers and with high antioxidant properties have been obtained.

LWT-Food Science and Technology

20th January 2014

Dear Sirs,

We enclose the manuscript entitled **“Wild edible plants as potential antioxidant or nutritional supplements for beverages minimally processed”**, for its possible publication in **LWT-Food Science and Technology** as an original full paper.

First of all we would like to thank the reviewers for their time to thoroughly review the manuscript and for their comments and suggestions in order to improve it. Specific responses to reviewers' comments are detailed in the “Detailed Responses to Reviewers” attached. We also indicate the modifications introduced in the manuscript according to the reviewers' recommendations.

We think this paper in its revised version could be adequate for publication in this journal and we thank you in advance for your interest. Once more we would like to thank the reviewers for their helpful suggestions to improve our manuscript.

I am looking forward to hearing from you

Yours sincerely,

M Teresa Pretel Pretel

Reviewers' comments:

Reviewer #1:

Highlights

-Wild edible plants incorporated to orange and kiwi juices increased antioxidant capacity

- 2nd OK

- 3rd OK

The highlight n°1 has been changed.

Introduction

ln. 52, high antioxidant capacity.

The word has been changed

Methods

ln. 104. proportion of 0.5% w/v. What is the criteria for the selections of this %? previous experiments (cited?) previous publications (cited?)

Previous experiments performed in our laboratory adding 0.1, 0.5, 1 and 2% of WEP, showed that the addition of 0.5% to orange juice and kiwifruit cremogenate, obtained the best organoleptic results. Thus this was the criteria followed. We added this explanation in material and methods section (lines 103-109).

ln. 132-136

Determination of vitamin C (AA + DHAA) using Egea et al. method. Please, describe briefly. Only a colorimetric method? (difficult to really quantify AA and DHAA) or an HPLC-based method?

Even if briefly described, some detail of the method should be included, for the replicability of the experiments and to avoid misinformation or missguide to readers if the access to the citation is not possible.

The paragraph has been re-writted in material and methods section (lines 133-146)

Results and discussion

AA+DHAA results. Vitamin C, how was determined and quantified (to be sure that both forms were detected/measured). In order to clearly differentiate between the vitamin C results from the ascorbic acid results.

We explained the methodology to determine and quantify ASC and DHA in material and methods (lines 133-146)

ln. 324 glycosides. *The word has been changed in the text*

Reviewer #2:

In this manuscript, authors present data on the addition of some edible plants to obtain new beverages with enhanced functional properties. The conducted research is considered as very innovative and will contribute to the knowledge about fruit juices. The experiments were well established with proper statistical analysis, as well as the used methodology for claiming the results. Then, I consider that the manuscript could be accepted after minor revisions.

Highlight nº 3. It needs to re-write

The resulted new beverages were even more accepted by the sensory panel that the fresh product.

The highlight nº3 has been changed.

Introduction

Authors should add at the end of this section the innovative and relevance of the addition of the edible plants with antioxidant properties for the juice fruit industry.

The comment has been added (lines 80-89).

Table 1.

Column 1. Change Matriz with WEP by "Matrix".

The word has been changed in the table.

References.

Serrano et al. 2009. Some surnames of the authors are missed. Check this reference.

The reference has been corrected.

Editor's comments:

General comments:

1.The international system of units (SI) should be used. If other units are mentioned, please give their equivalent in SI.

- Concentrations:

- "%", "ppm" is not accepted for concentration or moisture content in text, figures and tables. Use directly measured units, such as g moisture/g dry solids or g/100g or ml/100ml. Please refrain from using % (v/v), % (w/v) as well.

- "N" for normal concentration and "M" for molar should be changed to mol equi/L and mol/L, respectively.

Units in the text have been corrected.

2.When referring to equipment and materials please provide full information regarding manufacturer, code or brand, City, Country).

Text has been changed according to the comments.

Specific comments

1.line 89. Spell out "SE", *The word has been spelled out (line 89-90)*

2. line 92 Maturity of fruits? *See the answer above*

3. Section 2.2 Where were fruits obtained from? Maturity?

Kiwifruit of variety Hayward (Actinidia deliciosa) from Galicia (España) was employed. For the making of orange juice (OJ), oranges of variety Navel Lane-Late (Citrus sinensis) from Valencia (España) were employed. Both kiwi and orange fruits were purchased at commercial maturity state. We added this explanation in material and methods section (lines 105-108)

4. line 110 and 111. Please provide more information about the panel: age range, gender distribution, training.

What does "some expertise in testing other foods" refer to?

Sensory evaluation was conducted by a selected and trained panel comprising ten judges in accordance with ISO 8586-1:1993. For sake of clarity we changed material and methods at this point.

Table 1

Tables should be understandable without having to refer to the text or references.

Please indicate how the numbers were determined.

Table 1 has been changed.

Table 2 and 3

Please add # of replications (n ?)

Number of replications has been added in tables 2 and 3

Figure 1 and 2

Please remove the key explaining the color of the bars from the figure itself and move this information into the figure legend.

The number of replications should be stated (n ?)

Number of replications has been stated in material and methods (page 5 lines 189-190)

The figures and figure captions have been changed.

**Wild edible plants as potential antioxidant or nutritional supplements for beverages
minimally processed**

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Highlights

- Wild edible plants incorporated to orange and kiwi juices increased antioxidant capacity
- The addition of wild edible plants to beverages minimally processed improved the content in phenolic compounds and the antioxidant capacity.
- The resulted new beverages were even more accepted by the sensory panel that the fresh product.

Wild edible plants as potential antioxidant or nutritional supplements for beverages minimally processed

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ABSTRACT

Wild edible plants (WEP) with high antioxidant capacity, *Rosa canina* fruits, *Quercus ballota* acorns and *Sanguisorba minor* (young leaves and stems), were added to orange and kiwifruit juices for increase the nutritional properties. The addition of WEP to orange juice (OJ) and kiwifruit cremogenate (KC) does not affect, or it can even improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued. The beverages minimally processed with higher TSS were the matrices fortified with *Q. ballota*, the same that were considered sweetest by the judges. The addition of WEP to OJ did not significantly change the levels of vitamin C (ascorbic acid plus dehydroascorbic acid). The addition of *R. canina* and *S. minor* increased the content of phenolic compounds and antioxidant capacity in both matrices (OJ and KC) in more than 30 %. Beverages with a high interest for consumers and with high antioxidant properties have been obtained.

Keywords: wild edible plants, nutritional supplement, antioxidant potential, fruit, minimal processing

1. Introduction

During the last years, both food quality and its effects on human health has become a fundamental issue. As a result, many different research works about the functional properties of different foods, plant extracts and natural ingredients have been developed (Serrano et al., 2009). This new awareness have taken food industry to focus the attention on the development of functional or nutraceutical foods with beneficial properties for health (Lavecchia, Rea, Antonacci & Giardi, 2013). Some years ago, the compounds with nutraceutical activity added to foods were usually chemically produced, but new demands for a sustainable life have made that the food industry is now using natural compounds, mainly those derived from plants. Therefore, we can now find in the market a great variety of functional products consisting of food matrices to which extracts of botanical species with known functional and nutraceutical properties have been added (González-Molina, Moreno, & García-Viguera, 2008).

In this regard, there is a great variety of wild non-cultivated plants that have been employed in the diet and the traditional medicine of the Mediterranean region (Pardo de Santayana, Tardío & Morales, 2005), but their use has now decreased, an even disappeared, as a consequence of the industrial revolution. Among them, *Rosa canina* L., *Quercus ballota* Desf. and *Sanguisorba minor* Scop., are good candidates for their study as possible functional ingredients due to the nutraceutical and therapeutic properties that many recent studies have found in them. *R. canina* is a significant source of tocopherols, flavonoids, carotenoids and ascorbic acid (Barros, Carvalho, & Ferreira, 2011) and this composition is probably the reason for its high antioxidant capacity (Egea, Sánchez-Bel, Romojaro & Pretel, 2010). *R.*

canina also presents anti-inflammatory (Lattanzio et al., 2011) and antibacterial activities (Yilmaz & Ercisli, 2011), anticholinesterase capacity (Boğa, Hacıbekiroğlu, & Kolak, 2011) and antidiarrhoeal properties (Mandade, Choudhury, Harsulkar & Wakade, 2011). On the other side, the fruits of *Q. ballota* are rich in α - and γ -tocopherol and phenolic compounds, especially hydrolysable tannins (Tejerina, García-Torres, Cabeza de Vaca, Vázquez & Cava, 2011). *Q. ballota* also presents a high antioxidant capacity (Tejerina et al., 2011; Romojaro, Botella, Obón & Pretel, 2013), and the antioxidant activity of the acorn powder has been even characterised in order to be used as a new natural antioxidant for fats and oils (Lee, Jeong & Oh, 1992). Regarding *S. minor*, it has been already shown that its young leaves and stems have many and varied phenolic compounds that are derivatives of caffeic acid, apigenin, quercetins, kaempferol with a high antioxidant and antimycotic activities (Gatto et al., 2011; Romojaro et al., 2013).

Fruit juices are usually employed as food matrices for the production of functional foods. In fact, recent studies have shown that the beneficial properties of fruit juices can be increased by adding antioxidant sources (Gonzalez-Molina et al., 2008). Orange (*Citrus cinensis*) is a common raw material for the world fruit juice industry and its juice has a high nutritional value due to bioactive compounds, as well as an appreciated flavour and aroma (Pala & Toklucu, 2013). The kiwifruit has also a great potential for industrial exploitation, mostly due to its composition rich in phytonutrients (Tavarini, Degl'Innocentia, Remorinib, Massaib & Guidi, 2008). However, the obtaining of a good quality processed kiwifruit is not simple and the possibility to have an industrial valorisation of the fruit is yet away to be found. The main goal of the kiwifruit processing is to obtain safe and stable products able to retain as much as possible the peculiarity of fresh fruit, including green colour, aroma, nutritional value and structural characteristics. One of the main limitations for obtaining kiwifruit minimally processed is the difficulty for maintaining the original green colour

which, due to oxidation, quickly acquires brownish tonalities (Cassano & Drioli, 2007; Benlloch-Tinoco, Igual, Rodrigo & Martínez-Navarrete, 2013).

Food industry is currently focused on the development of novel and minimally processed products with improved quality. This type of products has been traditionally preserved by means of conventional thermal technologies. However, it usually requires the use of high temperatures combined with long process times which has been widely associated with a marked deterioration in food quality, especially with the development of cooked off-flavours, colour alteration and the loss of thermosensitive compounds (Benlloch-Tinoco, et al., 2013). For this reason, alternatives to conventional processing technologies are being explored. The addition of extracts of wild plants with high antioxidant capacity could, apart from increasing the nutritional value of the juices, help to stabilize and even improve their valued organoleptic properties.

Considering all the previous background, the main aim is to study if the incorporation of *R. canina*, *Q. ballota* and *S. minor* to orange and kiwifruit juices minimally processed could increase the nutritional properties and stabilize or even improve their organoleptic characteristics.

2. Materials and methods

2.1. Wild Edible Plants (WEP)

The non-cultivated and traditionally collected wild edible plants were harvested in the South East (SE) of Spain. Plants were frozen in liquid nitrogen, lyophilized and powdered using a domestic mixer and, finally, stored at 20 °C until further analysis. Wild edible plants used in this study were *Rosa canina* (fruits), *Quercus ballota* (fruits) and *Sanguisorba minor* (young leaves and stems).

2.2. Fruit matrices

For the making of kiwifruit cremogenate (KC), kiwifruit of variety Hayward (*Actinidia deliciosa*) from Galicia (España) was employed. For the making of orange juice (OJ), oranges of variety Navel Lane-Late (*Citrus sinensis*) from Valencia (España) were employed. Both kiwi and orange fruits were purchased at commercial maturity state. The orange juice was obtained using a domestic squeezer (Princesa, Barcelona, Spain) and the kiwifruit cremogenate was obtained using a domestic blender (Osterizer, Madrid, Spain). Both fruits (kiwi and orange) were processed to make juice at eating ripeness.

2.3. Preparation of enriched beverages minimally processed

The lyophilised WEP were added to the fruit matrices: natural orange juice (OJ) and kiwifruit cremogenate (KC) and they were homogenised in polytron (Kinematica AG, Lucerne, Switzerland). Previous experiments performed in our laboratory adding 0.1, 0.5, 1 and 2 g of WEP per 100 mL of matrix, showed that the best organoleptic results were obtained when proportion of 0.5 g 100mL⁻¹ was used. Thus this was the proportion used in the subsequent experiments. Homogenised mixtures were filtered in a gauzed filter and stored at room temperature for 24 hours. Samples were analysed after filtering (time 0) and after 24 h of storage.

2.4. Sensory Determination

Sensory evaluation was conducted by a selected and trained panel comprising ten judges in accordance with ISO 8586-1:1993. The tasting room was kept at 20 °C and beverages served in tasting glasses certified and coded. The evaluation was done using 10-point structured scales, 10 being the best and 1 the worst quality (colour, acidity, sweetness and aroma). The general acceptance quality attribute was assessed as the measurement of the acceptability of

the product by the consumer using a scale from “very unpleasant” (level 1) to “very pleasant” (level 10). The selection and training of tasters, as well as the fitting-out of the tasting room, were carried out according to Sanchez-Bel, Egea, Romojaro & Martinez-Madrid (2008). The purchase intent was also evaluated. Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

2.5. Quality parameters (TSS, pH, TA)

The total soluble solids content (TSS) was determined by measuring the refractive index of the juice from the fruit with a digital refractometer (Atago, Tokyo, Japan). TSS content was expressed as percentage. pH was measured in a pHmeter (Crison, Barcelona, Spain). Titratable acidity (TA) was determined by potentiometric titration with 0.1 mol equi L⁻¹ NaOH to pH 8.1. The results were expressed as grams of malic acid per 100 g of fresh weight.

2.6. Color quality

Colour was measured with a CR-400 colorimeter (Minolta, Paris, France) and results were expressed in CIELAB units of L*, a* and b*. Hue angle (H) and chroma (C) were computed from L*, a* and b* as $H = \tan^{-1}(b^*/a^*)$ and $C = \sqrt{(a^*)^2 + (b^*)^2}$.

2.7. Determination of vitamin C (ascorbic and dehydroascorbic acid)

Fruit ascorbate (ASC) content was determined according to Egea et al. (2010) with some modifications; three millilitres of samples homogenised in 10 mL of 5 g 100 mL⁻¹ cold metaphosphoric acid and stirred continuously for 30 min at 4 °C in darkness, and then centrifuged at 20,000 g for 25 min. The supernatant was passed through a C18 column (Sep-pack plus, Waters, Barcelona, Spain) and a 0.2 µm filter. The filtered supernatant was used for ascorbic acid determination using HPLC (LC-10Atvp, Shimadzu, Barcelona, Spain) with a

thermostated ion-exchange column (ION-300) at 30 °C and isocratic elution. The absorbance was recorded with a UV/vis detector at 245 nm. A standard curve in the range 10-100 mg kg⁻¹ ascorbic acid was used. The results were expressed as grams of ascorbic acid per 100 g of fresh weight.

Total ascorbate content was determined after reduction of dehydroascorbate (DHA) to ASC with DTT in a final concentration of 30 mmol L⁻¹ for 8 hours in the dark, and then measured as ASC following re-chromatography. Concentration of DHA was estimated from the difference between total ascorbate pool (ASC plus DHA) and ASC.

2.8. Determination of total phenolic compounds

Total phenolic compounds were determined according to Serrano et al. (2009) using (3 g) homogenized in 10 mL of 100 mmol L⁻¹ sodium phosphate buffer containing 150 mmol L⁻¹ NaCl, pH 7.4 on ice. The concentration of the total phenolic compounds in fruit extracts was determined by comparison with the absorbance of analytical grade phenol standard, gallic acid, at different concentrations. The results were expressed as grams of galic acid per 100 g of fresh weight (FW).

2.9. Determination of antiradical activity: Trolox equivalent antioxidant capacity (TEAC assay)

The ABTS[•] radical anion solution was generated by incubating, at 60 °C for 6 min, a mixture of 2.5 mmol L⁻¹ 2,2'-azobis(2-amidinopropane) hydrochloride (ABAP) and 20 mmol L⁻¹ 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS²) stock solution in 100 mL of phosphate buffer (100 mmol L⁻¹ phosphate and 150 mmol L⁻¹ NaCl, pH 7.4). Samples (3 g) were homogenized in 10 mL of 100 mmol L⁻¹ sodium phosphate buffer containing 150 mmol L⁻¹ NaCl, pH 7.4 on ice. The supernatant was collected as final extract. 40 µL of each extract

were mixed with 1960 μL of the radical solution to measure the antioxidant activity at 734 nm after 6 min of incubation at 36°C. A calibration curve was prepared with different concentrations of Trolox. The TEAC activity was calculated according to Egea et al. (2010) and represents the concentration of Trolox, in $\mu\text{mol L}^{-1}$, that has the same antioxidant capacity as the analyzed sample (Trolox equivalent).

2.10. Statistical analysis

Tests for significant differences were carried out using the general linear model of the SPSS (version 14.0, SPSS Inc., Chicago, USA) statistical package. Analysis of variance (ANOVA) was conducted using the treatments as a statistical parameter, the confidence level being 95 %. Correlations between different parameters were computed as Pearson's correlation coefficient (r). When differences were significant, multiple comparisons test was made using least significant difference test (LSD). Values represent the means $\pm$ SD of three replications for each of three sub-samples per sample.

3. Results and discussion

3.1 Consumers' acceptance

The sensory assessment is the main aspect for designing new foods because changes in aroma, texture or colour of the original matrices can significantly affect the consumer's acceptance. Although the overall impression is a function of many attributes and their interactions, it is interesting to identify the particular attributes that move acceptance. The complexity of food is such that it may well be impossible to truly discern the relative contributions of the different sensory inputs to food acceptance (Janzantti, Machado & Monteiro, 2011). In this work, the attribute colour was assessed in different ways according to

the matrix (OJ or KC) and the added WEP (*Rosa canina* -*Rc*-, *Quercus ballota* *Qb*- or *Sanguisorba minor* *Sm*-) (Table 1). The colour of the OJ fortified with *Rc* was significantly ($p<0.05$) better valued than the control one (119.72 ± 5.31), while the addition of *Qb* did not present differences regarding control OJ (95.87 ± 3.73). The addition of *Sm* to OJ reduced the redness of the orange juice and this fact was negatively assessed by the consumer (39.14 ± 10.59). Regarding KC, consumers positively assessed the increase of green colour intensity provided by *Sm* (124.01 ± 8.73). On the contrary, the addition of *Rc* gave KC a brownish aspect that was significantly worse valued (61.86 ± 9.42) than that of control KC. All matrices were considered less acid than control, regardless of the added WEP. The WEP addition increased the sweetness between 35 and 85 % regarding the unfortified matrices. The addition of WEP to OJ and KC decreased the aroma assessment in most mixtures, although not significantly. In our work, and in keeping with other authors (Tepper, 1993) regarding overall impression, colour was the most significant value because the worst assessed mixtures were OJ with *Sm* and KC with *Rc*, a fact coincident to the worst assessment of colour. The best valued were OJ with *Rc* and KC with *Sm*. Consumers showed a better purchase intent for OJ with *Rc* and KC with *Sm* and *Qb*.

It is worth mentioning the high purchase intent of KC with *Qb* because it would be expected that this enriched beverage show an increase of an astringent and bitter flavour due to the high content of tannins. However, matrices with *Qb* obtained a better overall impression and purchase intent than KC. Some authors have stated that the astringent flavour can be modified by the addition of other compounds, and that the sweetness can reduce the astringent sensation. In this study, matrices with *Qb* were those obtaining the highest punctuation in sweetness, a fact stating that, despite the high content in tannins of *Qb*, the extra supply of sweetness can avoid the astringent and bitter flavour of the tannins. In general, it could be concluded that the addition of WEP to OJ and KC does not affect, or it can even

improve, some parameters of consumer's acceptance, although the mixtures increasing sweetness and intensifying the matrix colour without changing the tone are the best valued.

3.2 Changes in quality parameters

The addition of WEP to matrices (OJ and KC) did not significantly change ($p<0.05$) the pH or the TA of the mixtures (Table 2). The TA of the new beverages ranged between 1.66 ± 0.04 of OJ with *Sm* and 1.34 ± 0.04 of KC with *Sm*. The pH of the mixtures remained between 3.5 and 4.25, within the levels found by other authors (Barros et al., 2011). However, the TSS with WEP was higher in all cases than in control matrices, and they coincide with the sweetness value given by judges. Therefore, while the TSS of the control OJ did not exceed 10.67 °Brix, when the juice was fortified with WEP this parameter was higher, ranging between 11.11 ± 0.15 in freshly made OJ with *Sm* and 11.83 ± 0.11 in OJ with *Qb* after 24 hours of storage at 20 °C. The TSS of KC was also higher in matrices enriched with WEP than in control KC. While TSS of control KC did not exceed 13.93 °Brix, the TSS of the fortified mixtures of kiwifruit ranged between 14.30 ± 0.15 °Brix of freshly made KC with *Sm* and 14.94 ± 0.24 of KC with *Qb* after 24 hours of storage at 20 °C. In general, the beverages minimally processed with higher TSS were the matrices fortified with *Qb*, the same that were considered sweetest by the judges (Table 2). This increase of TSS is an interesting characteristic because it can increase the sweetness of the beverages without adding sugars and, therefore, avoiding the increase of empty calories (Guenther, Reedy & Krebs-Smith, 2008), a more and more demanded issue by consumers.

3.3 Changes in colour quality

The enrichment of OJ and KC with WEP with high antioxidant capacity can change colour, although it can also contribute to its stability against oxidation. In OJ, the addition of *Rc*

significantly increased ($p<0.05$) the orange colour of the juice by increasing the redness (CIE a^* value) and the yellowness (CIE b^* value) (Table 3). On the contrary, the addition of *Qb* did not change the CIE a^* , although it increased the lightness (CIE L^* value) and decreased the intensity of the orange colour. The addition of *Sm* significantly changed ($p<0.05$) colour (CIE a^* more negative than that of control) due to the chlorophyll of leaves and young shoots of this plant that gives the juice a colour less valued by consumers (Table 2). It is worth mentioning that the addition of *Rc* to KC gave a brownish colour to the mixture (CIE a^* -4.53 ± 0.04 and Hue angle 105.36 ± 0.09), coincident with a low value given by consumers (61.87 % regarding control). However, the resulting colour of the mixture of *Qb* or *Sm* with KC, with lower CIE a^* and CIE b^* values, was better valued by consumers than that of control (Table 2). The mixtures of different matrices change the profile of the pigments (Lemmens, Van Buggenhout, Van Loey & Hendrickx, 2010). So WEP with high contents of chlorophyll and brown pigments are more suitable for KC, while those rich in carotenoids (orange and red pigments) are more suitable for OJ in order to obtain a product well valued by consumers.

After 24 hours at 20 °C, the orange colour of OJ was significantly ($p<0.05$) less intense, as it can be shown by the decrease of CIE a^* and CIE b^* (Table 3) probably caused by the degradation of β -cryptoxanthin, antheraxanthin and violaxanthin, the main responsible carotenoids for the colour of the orange juice (Carmona, Zacarias & Rodrigo, 2012). Although the addition of *Rc* to OJ did not avoid the degradation of carotenoids of the juice, the OJ with *Rc* preserved an intense red colour due to the increase in the mixture of β -carotene found in *Rosa canina* (97.77 mg 100 g⁻¹ DW) (Barros et al., 2011). It is already known that the stability of red colour is a big problem in food processing (Rein & Heinomen, 2004) and, therefore, the addition of *Rc* as colouring agent to certain matrices could compensate for the loss of pigments during the processing and could enrich the juices with

other bioactive compounds found in *R. canina* (Egea et al., 2010; Barros et al., 2011). On the other side, the addition of *Qb* to the OJ implied a quicker loss of red colour of the OJ regarding control, while the OJ with *Sm* practically reached the same colour than the control one, it won redness (less negative CIEa* value). Therefore, it seems that *Qb* potentiates the degradation of the characteristic carotenoids of the orange juice and the OJ potentiates the degradation of the chlorophyll of *Sm*.

The colour of KC changed after 24 hours at 20 °C, with a significant decrease of the Hue angle (more than 70° of difference between initial and final values), a fact showing a high percentage of darkening (Gonzalez-Molina et al., 2008). The addition of WEP to KC affects in different ways according to the species. While the addition of *Sm* does not reduce darkening (Hue angle value similar to the control), the addition of *Rc* and *Qb* significantly reduce darkening of KC and, particularly, the addition of *Rc* reduce darkening to half. Therefore, *Qb* and *Rc* could be used as an alternative to synthetic compounds for minimizing the darkening in other products that are minimally processed like vegetables cremogenate. However, the use of these species as anti-darkening agents would need a deeper study because the colour stabilization depends upon the structure and concentration of the pigment, pH, temperature, presence of co-pigments, ascorbic acid, metallic ions, etc (Wicklund et al., 2005).

3.4 Changes in Vitamin C and polyphenols contents

The addition of WEP to OJ did not significantly change ($p<0.05$) the levels of vitamin C (ascorbic acid plus dehydroascorbic acid) regarding the non fortified OJ and KC matrices (Figure 1a, c). The initial levels of vitamin C in OJ were 124.75 ± 3.35 mg 100 g⁻¹ FW, similar to those found by other authors (Tarrago-Trani, Philips & Cotty, 2012). Although the addition of WEP increases vitamin C between 10 and 15 %, it was not a significant increase. Vitamin

C in KC did not change regarding control when WEP were added; the levels ranged around 170 mg 100g⁻¹ FW, similar to those found by other authors (Celik, Sezai & Turgut, 2007). The stability of vitamin C in matrices (OJ and KC) at 20 °C during 24 hours did not significantly change ($p<0.05$). So, in general, the addition of WEP to OJ and KC did not significantly change the content of vitamin C or increase its stability under tested conditions. For nutritional purposes, vitamin C is the sum of AA and dehydroascorbic acid (DHAA); DHAA absorbed from the intestinal tract undergoes intracellular reduction (via NADPH- and glutathione-dependent reductases), rendering it biologically active (Gonzalez-Molina et al., 2008). It must be mentioned that when the matrix is OJ, the proportion of AA (Figure 1b) after 24 hours at 20 °C significantly increased ($p<0.05$). AA is susceptible to oxidation in aqueous solution; therefore, temperature, light, pH, the presence of oxygen, metal catalysts (iron, copper) and enzymes such as ascorbate oxidase affect the rate of AA degradation, at a variable rate in different foods (Johnston, Steinberg & Rucker, 2007). It was verified (figure 1b embedded) that, in aqueous solution and under tested conditions (24 hours at 20 °C), the speed of degradation of the ascorbic acid is very high during the first hours, regardless the concentration of AA of the initial solution.

The ascorbic acid in OJ or KC (Figure 1b, d) did not significantly degrade ($p<0.05$) after 24 hours at 20 °C. The TA and the pH of the matrices contribute to the AA stability because at low pH AA is fully protonated and relatively stable, with maximal stability at 4-6; at pH >6, AA is reversibly oxidized to DHAA, and DHAA may be further irreversibly oxidized to 2,3-diketo-l-gluconic acid, followed by degradation to other by-products (Johnston et al., 2007). On the other side, OJ is a rich source of flavanons, including hesperidin and narirutin, which are also main phenolic compounds of OJ (Proteggente et al., 2002). Moreover, kiwifruit is rich in polyphenols compared with other fruits (Park et al., 2006). The addition of *Rc* and *Sm* increased the content of phenolic compounds in both

matrices (OJ and KC) in more than 30 % due to the fact that both *Rc* and *Sm* have a high concentration of these compounds (Barros et al., 2011; Egea et al., 2010). However, the addition of *Qb* did not significantly change the levels of polyphenols (Figure 2 a, b). The high concentration of tannins found in *Qb* could cause the precipitation of tannin acids or tannin-protein complexes responsible for the astringent flavour of the tannins, a flavour that is disgusting to the consumer (Tejerina et al., 2011). The phenolic compounds remained stable during 24 hours at room temperature in all matrices.

3.5. Changes in Trolox Equivalent Antioxidant Capacity (TEAC) and relationship with phytonutrient levels

One of the main quality attributes of juices is their antioxidant capacity and, during the last years, this has been a very solid argument for the professionals of the food industry. Figure 2 (c and d) shows the antioxidant capacity (TEAC) of OJ and KC, respectively, after their enrichment with different WEP. The initial antioxidant capacity of KC was higher than that of OJ (359.04 ± 46 and 298.07 ± 10.2 equi Trolox $\mu\text{mol L}^{-1}$, respectively). There is a controversial debate in the literature about the influence of vitamin C on the antioxidant capacity of fruits or vegetables (Guo et al., 2003; Tavarini et al., 2008). It is also known that fruits with high antioxidant capacity generally contain more antioxidants and most of these antioxidants has been showed to be phenolic compounds and, in particular, flavonoids (Guo et al., 2003). According to some authors (Proteggente et al., 2002; Tavarini et al., 2008), the antioxidant capacity of OJ mainly results from its ascorbic acid content, while flavanone glycosides have a limited contribution to its antioxidant capacity. In our work, the addition of *Qb* did not significantly increase the antioxidant capacity of the matrices (OJ and KC), while the addition of *Rc* increased in 67 and 39 % the antioxidant capacity of OJ and KC, respectively. The antioxidant capacity of OJ and KC with *Sm* also increased in 60 and 30 %, respectively.

Coincident to our results, some studies have shown that the activity in the TEAC assay increases with the number of hydroxyl groups of the antioxidant compound, so the antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts under experimental conditions (Hodisan, Socaciu, Ropan, & Neamtu, 1997). This relation between total phenols and the antioxidant activity has been found in many fruits and all their derived products (Egea et al., 2010), a fact suggesting that the phenolic compounds could be responsible for the antioxidant properties of the new beverages fortified with *Rc* and *Sm* because there is a good correlation (Table 4) between the content in polyphenols of the new beverages and the antioxidant capacity and, however, there is a very low correlation between the content in vitamin C and the antioxidant capacity of the mixtures.

However, when the new beverages were stored during 24 hours at 20 °C the antioxidant capacity of the beverages fortified with *Rc* and *Sm* significantly decreased, between 10 and 15 % in OJ and between 15 and 25 % in KC. This decrease could be due to reactions of polymerization, condensation or complexation that can cause the formation of new compounds with a lower antioxidant capacity (Gonzalez-Molina et al., 2008). Moreover, the presence of other antioxidant compounds from *Rc* and *Sm*, such as proteins, which we have not analyzed, could react in the mixtures decreasing the in vitro antioxidant values (Gonzalez-Molina et al., 2008). The antioxidant properties of food matrices are due to the presence in complex mixtures of compounds of varying polarity, such as vitamin C, vitamin E, carotenoids and polyphenols. Due to the potential synergistic action of all these bioactive compounds present in food (Egea et al., 2010) the total antioxidant capacity of some foods has increasingly become a sales argument for professionals in the food industry.

4. Conclusion

The beverages that result from the addition of wild edible plants with high antioxidant capacity to orange juice or kiwifruit cremogenate are well accepted by consumers, even improving the acceptance of the fresh product, and they improve the content in phenolic compounds and the antioxidant capacity without changing the physico-chemical properties of the matrices. This better acceptance of enriched beverages minimally processed by the consumer was due, to a great extent, to the intensification of the natural colour (in the case of addition of *Rc* to OJ and *Sm* to KC) and to the extra sweetness provided mainly by *Qb* and, to a lesser extent, by *Rc* because the addition of wild edible plants to the matrices increased their content in soluble solids. Beverages minimally processed with a high interest for consumers and with high antioxidant properties have been obtained.

References

- Barros, L., Carvalho, A.M. & Ferreira, I. (2011). Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. *Food Research International*, 44 (7), 2233–2236.
- Benlloch-Tinoco, M., Igual, M., Rodrigo, D. & Martínez-Navarrete, N. (2013). Comparison of microwaves and conventional thermal treatment on enzymes activity and antioxidant capacity of kiwifruit puree. *Innovative Food Science and Emerging Technologies*, 19, 166-172.
- Boğa M., Hacıbekiroğlu, I. & Kolak, U. (2011). Antioxidant and anticholinesterase activities of eleven edible plants. *Pharm Biol*, 49(3), 290-295.
- Carmona, L., Zacarias, L., Rodrigo, M.J. (2012). Stimulation of coloration and carotenoid biosynthesis during postharvest storage of ‘Navelina’ orange fruit at 12 °C. *Postharvest Biology and Technology*, 74, 108–117.

401 Cassano, A. & Drioli, E. (2007). Concentration of clarified kiwifruit juice by osmotic
 402 distillation. *Journal of Food Engineering*, 79, 1397-1404.

403 Celik, A., Sezai, E. & Turgut, N. (2007). Some physical, pomological and nutritional
 404 properties of kiwifruit cv. Hayward. *International Journal of Food Sciences and*
 405 *Nutrition*, 58, 411-418.

406 Egea, I., Sánchez-Bel, P., Romojaro, F. & Pretel, M.T. (2010). Six edible wild fruits as
 407 potential antioxidant additives or nutritional supplements. *Plant Foods For Human*
 408 *Nutrition*, 65, 121-129.

409 Gatto, M.A., Ippolito, A., Linsalata, V., Cascarano, N.A., Nigro, F., Vanadia, S., Di Venere,
 410 D. (2011). Activity of extracts from wild edible herbs against postharvest fungal
 411 diseases of fruit and vegetables. *Postharvest Biology and Technology*, 61 (1), 72-82.

412 González-Molina, E., Moreno, D.A. & García-Viguera, C., (2008). Aronia-enriched lemon
 413 juice: a new highly antioxidant beverage. *J Agric Food Chem*, 56, 11327-11333.

414 Guenther, P.M., Reedy, J. & Krebs-Smith, S.M. (2008). Development of the healthy eating
 415 index. *J Am Diet Assoc*, 108(11), 1896-901.

416 Guo, C.J., Yang, J.J., Wei, J.Y., Li, Y.F., Xu, J., Jiang, Y.G., 2003. Antioxidant activities of
 417 peel, pulp and seed fractions of common fruits as determined by FRAP assay.
 418 *Nutrition Research*, 23, 1719-1726.

419 Hodisan, T., Socaciu, C., Ropan, I., Neamtu, G. (1997). Carotenoid composition of *Rosa*
 420 *canina* fruits determined by thin-layer chromatography and high-performance liquid
 421 chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 16, 521-528.

422 Janzantti, N.S., Machado, T.V. & Monteiro, M. (2011). Sensory acceptance of juice from
 423 FCOJ processing steps. *Journal of Sensory Studies*, 26 (5), 322-330.

Johnston, C.S., Steinberg, F.M., Rucker, R.B. (2007). Ascorbic acid. In J. Zempleni, R.B. Rucker, D.B. McCormick, J.W. Suttie (Eds.), *Handbook of Vitamins* (4th ed., pp. 489-520). CRC Press, Boca Raton, FL, USA.

Lavecchia, T., Rea, G., Antonacci, A. & Giardi, M.T. (2013). Healthy and adverse effects of plant-derived functional metabolites: The need of revealing their content and bioactivity in a complex food matrix. *Critical Reviews in Food Science and Nutrition*, 53, 198-213.

Lattanzio, F., Greco, E., Carretta, D., Cervellati R., Govonic, P. & Speronia, E. (2011). In vivo anti-inflammatory effect of *Rosa canina* L. extract. *Journal of Ethnopharmacology*, 137: 880-885.

Lee, M.H., Jeong, J.H. & Oh, M.J. (1992). Antioxidative activity of gallic acid in acorn extract. *Journal of the Korean Society of Food and Nutrition*, 21(6), 693-700.

Lemmens L., Van Buggenhout, S., Van Loey, A.M. & Hendrickx, M.E. (2010). Particle size reduction leading to cell wall rupture is more important for the β -carotene bioaccessibility of raw compared to thermally processed carrots. *J. Agric. Food Chem.* 58, 12769-12776.

Mandade, R.J., Choudhury, A., Harsulkar, A. & Wakade, R. (2011). Role of the *Rosa canina* L. leaf extract as an antidiarrheal drug in rodents. *Indian Journal of Pharmacology*, 43, 316-319.

Pala, Ç.U. & Toklucu, A.K. (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT - Food Science and Technology*, 50(2), 426-431.

Pardo de Santayana, M., Tardío, J. & Morales, R. (2005). The gathering and consumption of wild edible plants in the Campoo (Cantabria, Spain). *Int J Food Sci Nutr*, 56(7): 529-542.

449 Park, Y.S., Junj, S.T., Kang, S.G., Drzewiecki, J., Namiesnik, J., Haruenkit, R., Barasch, D.,
 450 Trakhtenberg, S. & Gorinstein, S. (2006). In vitro studies of polyphenols, antioxidants
 451 and other dietary indices in kiwifruit (*Actinidia deliciosa*). *Int. J. Food Sci. Nutr*, 57,
 452 107 122.

453 Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E. & Wiseman, S.
 454 (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects
 455 their phenolic and vitamin C Composition. *Free Radical Research*, 36(2), 217-233.

456 Rein, M.J. & Heinonen, M. (2004). Stability and enhancement of berry juice color. *J. Agric.*
 457 *Food Chem*, 52(10), 3106-3114.

458 Romojaro, A., Botella, M.A., Obón, C. & Pretel, M.T. (2013). Nutritional and antioxidant
 459 properties of wild edible plants and their use as potential ingredients in the modern
 460 diet. *Int J Food Sci Nutr*, 64(8): 944-952.

461 Sanchez-Bel, P., Egea, I., Romojaro, F. & Martinez-Madrid, M.C. (2008). Sensorial and
 462 chemical quality of electron beam irradiated almonds (*Prunus amygdalus*). *LWT-Food*
 463 *Science and Technology*, 41, 442 449.

464 Serrano, M., Díaz-Mula, H.M., Zapata, P.J., Castillo, S., Guillén, F., Martínez-Romero, D., Valverde,
 465 J.M. & Valero, D. (2009). Maturity stage at harvest determines the fruit quality and
 466 antioxidant potential after storage of sweet cherry cultivars. *J Agric Food Chem*, 57, 3240-
 467 3246.

468 Tarrago-Trani, M.T., Philips, K.M. & Cotty, M. (2012). Matrix-specific method validation for
 469 quantitative analysis of vitamin C in diverse foods. *Journal of Food Composition and*
 470 *Analysis*, 26, 12-25.

471 Tavarini, S., Degl'Innocentia, E., Remorinib, D., Massaib, R. & Guidi, L. (2008). Antioxidant
 472 capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after
 473 storage of Hayward kiwifruit. *Food Chemistry*, 107, 282 288.

474 Tejerina, D., García-Torres, S., Cabeza de Vaca, M., Vázquez, F.M. & Cava, R. (2011).
 475 Acorns (*Quercus rotundifolia* Lam.) and grass as natural sources of antioxidants and
 476 fatty acids in the “montanera” feeding of Iberian pig: Intra- and inter-annual
 477 variations. *Food Chemistry*, 124, 997-1004.

478 Tepper, B. J. (1993). Effects of a slight color variation on consumer acceptance of orange
 479 juice. *Journal of Sensory Studies*, 8, 145-154.

480 Wicklund, T., Rosenfeld, H.J., Martinsen, B.K., Sundfør, M.W., Lea, P., Bruun, T., Blomhoff,
 481 R. & Haffner, K. (2005). Antioxidant capacity and colour of strawberry jam as
 482 influenced by cultivar and storage conditions. *Food Sci. Technol*, 38, 387-391.

483 Yilmaz, S.O. & Ercisli S. (2011). Antibacterial and antioxidant activity of fruits of some rose
 484 species from Turkey. *Romanian Biotechnological letters*, 16, 6407-6411.

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Figure captions

Figure 1: Changes in levels of total ascorbate (Vitamin C) and ascorbic acid content of orange juice (OJ), (figures a and b) and Kiwifruit cremogenate (KC) (figures c and d) fortified with WEP (*Rosa canina* -*Rc*-, *Quercus ballota* *Qb*- o *Sanguisorba minor* *Sm*-) after filtering, time 0 (□) or after 24 hours (■) at 20°C. Figure 1b embedded shows the degradation rate of ascorbic acid in aqueous solution, control (○), 20 (△), 40 (□) and 60 (◇) mg kg⁻¹ ascorbic acid under the same tested conditions as OJ and KC (24 hours at 20 °C). LSD (p<0.05), least significant difference, was used for significant difference comparison among means of samples.

Figure 2: Changes in levels of total polyphenol content (figures a and b) and Trolox Equivalent Antioxidant Capacity (TEAC) (figures c and d) of orange juice (OJ), (figures a and c) and Kiwifruit cremogenate (KC) (figures b and d) fortified with WEP (*Rosa canina* -*Rc*-, *Quercus ballota* *Qb*- o *Sanguisorba minor* *Sm*-) after filtering, time 0 (□) or after 24 hours (■) at 20°C. LSD (p<0.05), least significant difference, was used for significant difference comparison among means of samples.

Tables and Figures

Table 1: Consumer acceptance of orange juice (OJ) and kiwi liquefied (KC) after addition of wild edible plants (WEP). Results were expressed as percentage of acceptance being 100 the punctuation obtained by the unfortified juice matrix.

Matrix with WEP ^a	Colour ^b	Acidity ^b	Sweetness ^b	Aroma ^b	Overall impression ^b	Purchase intent ^b
OJ with <i>Rc</i>	119.72±5.31	87.36±5.81	153.57±17.45	84.76±12.42	103.45±8.35	117.62±9.79
OJ with <i>Qb</i>	95.87±3.73	92.08±7.04	184.72±31.93	83.57±6.08	100.87±9.40	95.24±5.53
OJ with <i>Sm</i>	39.14±10.59	85.92±7.95	155.56±35.44	80.95±11.28	80.00±24.34	89.76±18.6
KC with <i>Rc</i>	61.87±9.42	63.45±10.67	135.75±14.75	95.04±5.72	84.01±8.70	65.43±15
KC with <i>Qb</i>	114.34±6.97	84.64±10.76	157.01±30.06	82.54±10.78	125.1±15.5	115.00±14
KC with <i>Sm</i>	124.01±8.73	75.00±6.96	152.38±35.62	105.79±23.85	129.6±11.8	123.00±17
^c LSD (p<0.05)	11.67	10.67	40.44	17.31	29.09	15.17

^aMatrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* *Rc*, *Quercus ballota* *Qb* or *Sanguisorba minor* *Sm*).

^b Colour, acidity, sweetness, aroma, overall impression and purchase intent. (100 the punctuation obtained by the unfortified matrix).

^cLSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Table 2: Acidity, pH and soluble solids of orange juice (OJ) and kiwifruit cremogenate (KC) after addition of wild edible plants (WEP).

Matrix with WEP ^a	Time (h) ^b	TA (g 100g ⁻¹)	pH	TSS (Brix)
OJ Control	0	1.61±0.02	3.61±0.20	10.63±0.11
OJ with <i>Rc</i>		1.54±0.03	3.73±0.03	11.10±0.12
OJ with <i>Qb</i>		1.58±0.05	3.74±0.07	11.43±0.07
OJ with <i>Sm</i>		1.66±0.04	3.45±0.30	11.01±0.15
OJ Control	24	1.65±0.01	3.64±0.14	10.67±0.22
OJ with <i>Rc</i>		1.57±0.03	3.43±0.02	11.30±0.10
OJ with <i>Qb</i>		1.60±0.04	3.39±0.02	10.50±0.06
OJ with <i>Sm</i>		1.63±0.08	3.57±0.06	10.83±0.09
^c LSD (p<0.05)		0.06	0.20	0.18
KC Control	0	1.40±0.01	3.67±0.42	13.53±0.12
KC with <i>Rc</i>		1.41±0.06	4.07±0.13	14.78±0.38
KC with <i>Qb</i>		1.34±0.05	3.61±0.26	14.38±0.23
KC with <i>Sm</i>		1.34±0.04	4.25±0.26	14.33±0.07
KC Control	24	1.39±0.02	3.6±0.23	13.93±0.18
KC with <i>Rc</i>		1.44±0.03	3.74±0.29	14.87±0.30
KC with <i>Qb</i>		1.37±0.04	3.70±0.29	14.43±0.24
KC with <i>Sm</i>		1.35±0.03	3.64±0.25	14.30±0.15
^c LSD (p<0.05)		0.05	0.39	2.23

Values represent the means ± SD of three replications for each of three sub-samples per sample.

^a Matrix: Orange juice (OJ), kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* –*Rc*, *Quercus ballota* –*Qb* or *Sanguisorba minor* –*Sm*).

^b Time (0h and after 24h a 20°C).

^c LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Table 3: Colour of orange juice (OJ) and kiwifruit cremogenate (KC) after enrichment with wild edible plants (WEP).

Matrix with WEP ^a	Time (h) ^b	CIEL*	CIEa*	CIEb*	Croma	Hue
OJ Control		38.08±0.28	-5.58±0.04	18.47±0.45	19.30±0.44	106.83±0.27
OJ with <i>Rc</i>	0	39.82±0.19	-2.78±0.01	20.26±0.09	20.45±0.08	97.81±0.02
OJ with <i>Qb</i>		43.41±0.27	-5.38±0.03	22.37±0.31	23.01±0.31	103.53±0.15
OJ with <i>Sm</i>		38.22±0.18	-7.55±0.14	17.24±0.44	18.83±0.45	113.66±0.18
OJ Control		38.82±0.12	-5.92±0.12	17.92±0.37	18.87±0.39	108.29±0.21
OJ with <i>Rc</i>	24	39.58±0.33	-2.96±0.08	19.04±0.18	19.27±0.19	98.82±0.15
OJ with <i>Qb</i>		42.85±0.12	-6.15±0.19	20.80±0.53	21.70±0.53	106.49±0.47
OJ with <i>Sm</i>		38.74±0.78	-5.41±0.24	17.36±0.89	18.18±0.92	107.32±0.18
^c LSD (p<0.05)		0.49	0.18	0.65	0.66	0.33
KC Control		43.75±0.70	-7.41±0.08	16.12±0.22	17.74±0.22	114.69±0.32
KC with <i>Rc</i>	0	42.70±0.03	-4.53±0.04	16.48±0.21	17.09±0.22	105.36±0.09
KC with <i>Qb</i>		47.08±0.12	-7.76±0.05	18.06±0.20	19.60±0.21	113.32±0.11
KC with <i>Sm</i>		42.41±0.50	-9.14±0.17	18.75±0.12	20.86±0.15	115.98±0.38
KC Control		38.96±0.27	-2.77±1.23	11.39±0.38	19.25±1.00	44.64±1.82
KC with <i>Rc</i>	24	39.00±0.36	-2.27±0.27	13.24±0.26	32.34±7.32	71.84±12.81
KC with <i>Qb</i>		43.42±0.41	-4.42±0.10	13.26±0.62	34.29±7.61	62.81±16.06
KC with <i>Sm</i>		39.00±0.36	-2.27±0.27	13.24±0.26	18.26±1.31	48.49±11.03
^c LSD (p<0.05)		0.55	0.65	0.44	5.28	11.55

Values represent the means ± SD of three replications for each of three sub-samples per sample.

^a Matrix: Orange juice (OJ), Kiwifruit cremogenate (KC) fortified with WEP (*Rosa canina* -*Rc*, *Quercus ballota* -*Qb*, *Sanguisorba minor* -*Sm*).

^b Time (0h and after 24h at 20°C).

^c LSD (p<0.05), least significant difference, was used for significant difference comparison among means of various samples.

Table 4 Analisis of correlations between total antioxidant activity (TEAC), total poliphenols content and vitamin C in the prepared mixtures.

Antioxidant Capacity (TEAC)				
	OJ ^{0h}	KC ^{0h}	OJ ^{24h}	KC ^{24h}
Phenolics	0.9620 ^{MS}	0.9669 ^{MS}	0.9500 ^{MS}	0.9280 ^{MS}
Vitamin C	0.3095	0.5220	0.1988	0.2091

^{MS} Marginally significant ($P > 0.05$).

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548 Figure 1

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Figure 2

Research Article

Wild Edible Plants as Potential Antioxidants in Vegetables Oils

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The effect of the addition of fruits of *Rosa canina* and *Quercus ballota* and leaves of *Sanguisorba minor* to sunflower, seed, and olive oils on lipid oxidation during the frying process was analyzed. The three underutilised edible plants increased the oxidative stability of the sunflower oil, since the malondialdehyde (MDA) content was significantly decreased with respect to values of the control sunflower oil after the heating process. However, in olive oil, the effect of these edible plants on decreasing the lipid peroxidation was only evident for the highest concentration, while in seed oil the addition of the edible plant showed a prooxidant effect. Thus, these wild edible plants could be used to enrich vegetable oils with low content of natural antioxidant, such as sunflower oil, and avoid or decrease the use of synthetic antioxidant.

1. Introduction

Edible vegetable oils such as sunflower oil, seed oil, and olive oil have an important place in human diet, since their use in culinary processes is a constant. However, during frying, a gradual deterioration of oil quality occurs due to lipid oxidative decomposition reactions leading to lowering quality and nutritional value of foods, with consequences for the health of the consumer, causing aging, membrane damage, and numerous diseases, such as chronic inflammation, neurodegenerative diseases, adult respiratory distress syndrome, atherogenesis, diabetes, and different types of cancer [1]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ), are widely used in the food industry to avoid oxidation [2] due to their low cost and good performance, although their safety has been questioned due to carcinogenic effect [3]. According to the European Bulletin (order of 24 August 2007) [4] the maximum dose that allowed for these artificial additives in frying oils is 200 mg kg⁻¹ of oil.

Under these circumstances, research on development of safe natural antioxidants is therefore essential. Recently it

has been shown that *Nigella sativa* seed extract could be an interesting alternative to the use of synthetic antioxidants, since the addition of *Nigella* seed extract to sunflower oil improved their thermal stability and shelf-life [5]. Accordingly, Cordeiro et al. [6] have shown that extract of *Rosmarinus officinalis* added to sunflower, corn, and soybean oils displayed a more effective antioxidative action than TBHQ and it was stable at the frying temperature of the oils, unlike the majority of the antioxidants commonly used. Moreover, even in olive oils which contain high concentration of natural antioxidants, the addition of oregano essential oils leads to a protective effect on primary lipid oxidation [7].

Recent phytochemical studies have revealed that fruits from *Rosa canina* L. contain high amounts of vitamin C, carotenoids, and polyphenols making them a suitable source of antioxidants to be used commercially to retard rancidity in fatty materials in food manufacturing [8, 9]. Accordingly, acorns from *Quercus* spp. also possess biologically active substances, such as α - and γ -tocopherol and phenolics compounds, specially tannins, with proved antioxidant and free-radical-scavenging capacities [10, 11]. Finally, leaves and shoot from *Sanguisorba minor* Scop. (Sm) have also high

FIGURE 1: Lipid peroxidation value, expressed as malondialdehyde (MAD) concentration, in vegetable oils before the heating process (control) and after heating (AH) and after the heating process of these edible oils with the addition of several concentrations of the wild edible plants (WEP). For each vegetable oil ((a) sunflower, (b) olive, and (c) seed oil) data are the mean $\pm$ SE of two determinations performed in three replicates and LSD values are shown.

concentrations of phenolics compounds, the major being derivatives of caffeic acid, apigenin, quercetin, and kampherol [12].

Thus, the aim of this work was to evaluate the effect of the addition of dry powder from three underutilised edible plants, fruits of *Rosa canina*, corn of *Quercus ballota*, and shoots and leaves of *Sanguisorba minor* to sunflower, seed, and olive oils on lipid oxidation during the frying process.

2. Material and Methods

2.1. Wild Edible Plants (WEP) and Vegetable Oils. Fruits of *Rosa canina* L., acorns of *Quercus ballota* Desf., and leaves of *Sanguisorba minor* Scop. were harvested at their optimum stage for consumption according to traditional practices and transferred to laboratory. Then, 3 homogeneous samples (50 g) were randomly performed for each species,

which were frozen in liquid N₂, lyophilized, and ground to be used as additives to vegetable oils. The vegetable oils used were virgin olive oil from oil press of Jaen (Spain), sunflower oil (Savena-Ibérica, Sevilla, Spain), and special seed oil for frying with increased oleic acid content and E-900 antifoam (Hacendado, Mercadona, Spain). Samples of lyophilized WEP were added to the vegetable oils at 0.5, 1, and 5% (w : v) and the vegetable oils were heated at 100°C for 12 h. Experiments were performed in triplicate.

2.2. Lipid Peroxidation Measurement. Malondialdehyde (MDA) was quantified in duplicate in vegetable oils after the heating process as index of lipid peroxidation using the thiobarbituric acid reactive substrates (TBARS) assay [13]. Reaction mixture contained 1.5 mL 0.5% thiobarbituric acid (TBA) in 20% TCA and 0.5 mL of vegetable oil. The mixture was incubated at 90°C in a shaking water bath for 20 min, and the reaction was stopped by placing the reaction tubes in an ice-water bath. The samples were then centrifuged at 1000 g for 5 min, and the absorbance of supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mM cm⁻¹ and expressed as mmol MDA g⁻¹ of vegetable oil. The data are the means ± SE of quantifications made in duplicate in three replicates.

2.3. Statistical Analysis. Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were calculated using the statistical software (SPSS, version 14.0, SPSS Inc., Chicago, USA). Least significant difference values (LSD; $P \leq 0.05$) were calculated to find significant differences among treatments.

3. Results and Discussion

Peroxides and hydroperoxides are products that represent the beginning of lipoxidation, a step in which the chain-breaker antioxidants can interrupt the process and restore the fatty acid chain [14]. Then, hydroperoxides are spontaneously decomposed to MDA, which can be used as an indicator of lipid peroxidation damage [15]. MDA concentration increased in vegetable oils after the heating process, with important differences among the vegetable oil, and the addition of WEP showed different effect depending on plant species and vegetable oils (Figure 1). Thus, in sunflower oil the concentration of MDA increased from initial values of 2.24 ± 0.30 mmol g⁻¹ to 6.53 ± 1.63 mmol g⁻¹ after the heating process, this increase being significantly reduced by the addition of the three WEP. Moreover, this effect was similar to the three plant species and the three used concentrations (Figure 1(a)) and could be attributed to the high phenolics content of these plants [8–11]. Accordingly, the addition of *Nigella* seed extract to this vegetable oil increased its oxidative stability [5]. Similar effects have been reported in soybean oil enriched with white tea and olive leaves extract, in which the oxidative extension during microwave heating was reduced [16].

However, in olive and seed oils the heating process had no significant effect on MDA content (Figures 1(a) and 1(b)), probably due to the high natural antioxidant compounds present in virgin olive oil, the most important being phenolic compounds (phenolic alcohols and acids, flavonoids, lignans, and secoiridoids), carotenes, and tocopherols [17, 18], and to the synthetic antioxidant added to the seed oil special for frying. Moreover, the addition of these WEP powders to olive oil was effective in reducing lipid peroxidation when added at 5%, while at doses of 0.5 and 1% no significant effect was observed. Accordingly, the incorporation of rosemary, thyme, and lemon dried plants to olive oil just relatively helped to improve its thermal resistance [19]. On the contrary, in seed oil, the addition of WEP leads to different effect depending on plant species. Thus, MDA content decreased after the heating process with the addition of *Quercus ballota* corn, while the addition of increased concentration of *Rosa canina* fruit and leaves and shoots of *Sanguisorba minor* led to increases in MDA concentration, showing a prooxidant effect (Figure 1(c)). Other antioxidants have also different effects when added to different oils. Thus, TBHQ showed antioxidant effect in sunflower and soybean oils, while it displayed a prooxidant effect in corn oil [6].

Results show that the addition of dry powder of wild edible plants with high concentration of natural antioxidants could be an innovative and safety tool to increase thermal stability of vegetable oils such as sunflower, soybean, or corn and avoid or decrease the use of synthetic antioxidant.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Ana Romojaro and Paloma Sanchez-Bel contributed equally to this work.

References

- [1] M. D. Guillén and E. Goicoechea, "Toxic oxygenated α,β -unsaturated aldehydes and their study in foods: a review," *Critical Reviews in Food Science and Nutrition*, vol. 48, no. 2, pp. 119–136, 2008.
- [2] L. X. Qin, J. Chao, S. Yan, Y. M. Li, and C. X. Gang, "Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS," *Food Chemistry*, vol. 113, no. 2, pp. 692–700, 2009.
- [3] E. D. Frankel, *Antioxidants in Food and Biology. Facts and Fiction*, The Oily Press, Bridgwater, UK, 2007.
- [4] European Official Bulletin, BOE, (Order of 24 August 2007), BOE-A-2007-16339, http://www.boe.es/aeboe/consultas/bases_datos/doc.php?id=BOE-A-2007-16339.
- [5] F. Ammari, C. B. Y. Cordella, N. Boughanmi, and D. N. Rutledge, "The increase in oxidative stability of sunflower oil enriched with *Nigella sativa* L. seed extracts," *Food Measure*, vol. 6, pp. 12–20, 2012.
- [6] A. Cordeiro, M. L. Medeiros, N. A. Santos et al., "Rosemary (*Rosmarinus officinalis* L.) extract: thermal study and evaluation

- of the antioxidant effect on vegetable oils,” *Journal of Thermal Analysis and Calorimetry*, vol. 113, no. 2, pp. 889–895, 2013.
- [7] C. M. Asensio, V. Nepote, and N. R. Grosso, “Chemical stability of extra-virgin olive oil added with oregano essential oil,” *Journal of Food Science*, vol. 76, no. 7, pp. S445–S450, 2011.
- [8] L. Barros, A. M. Carvalho, and I. C. F. R. Ferreira, “Exotic fruits as a source of important phytochemicals: improving the traditional use of *Rosa canina* fruits in Portugal,” *Food Research International*, vol. 44, no. 7, pp. 2233–2236, 2011.
- [9] I. Egea, P. Sánchez-Bel, F. Romojaro, and M. T. Pretel, “Six edible wild fruits as potential antioxidant additives or nutritional supplements,” *Plant Foods for Human Nutrition*, vol. 65, no. 2, pp. 121–129, 2010.
- [10] A. Romojaro, M. A. Botella, C. Obón, and M. T. Pretel, “Nutritional and antioxidant properties of wild edible plants and their use as potential ingredients in the modern diet,” *International Journal of Food Science and Nutrition*, vol. 64, no. 8, pp. 944–952, 2013.
- [11] E. Cantos, J. C. Espín, C. López-Bote, L. de la Hoz, J. A. Ordóñez, and F. A. Tomás-Barberán, “Phenolic compounds and fatty acids from acorns (*Quercus spp.*), the main dietary constituent of free-ranged Iberian pigs,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 21, pp. 6248–6255, 2003.
- [12] M. A. Gatto, A. Ippolito, V. Linsalata et al., “Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables,” *Postharvest Biology and Technology*, vol. 61, no. 1, pp. 72–82, 2011.
- [13] R. L. Heath and L. Packer, “Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation,” *Archives of Biochemistry and Biophysics*, vol. 125, no. 1, pp. 189–198, 1968.
- [14] F. Shahidi and Y. Zhong, “Lipid oxidation and improving the oxidative stability,” *Chemical Society Reviews*, vol. 39, no. 11, pp. 4067–4079, 2010.
- [15] S. Y. Rogiers, G. N. M. Kumar, and N. R. Knowles, “Maturation and ripening of fruit of *Amelanchier alnifolia* Nutt. are accompanied by increasing oxidative stress,” *Annals of Botany*, vol. 81, no. 2, pp. 203–211, 1998.
- [16] R. Malheiro, N. Rodrigues, G. Manzke, A. Bento, J. A. Pereira, and S. Casal, “The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating,” *Industrial Crops and Products*, vol. 44, pp. 37–43, 2013.
- [17] D. L. García-González, R. Aparicio-Ruiz, and R. Aparicio, “Virgin olive oil—chemical implications on quality and health,” *European Journal of Lipid Science and Technology*, vol. 110, no. 7, pp. 602–607, 2008.
- [18] M. Servili, S. Esposto, R. Fabiani et al., “Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure,” *Inflammopharmacology*, vol. 17, no. 2, pp. 76–84, 2009.
- [19] M. A. Ayadi, N. Grati-Kamoun, and H. Attia, “Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants,” *Food and Chemical Toxicology*, vol. 47, no. 10, pp. 2613–2619, 2009.

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