UNIVERSITÀ degli STUDI di CATANIA
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Di3A

UNIVERSITÀ DEGLI STUDI DI CATANIA

AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCE

XXIX ciclo

New products from *Cynara cardunculus* L.

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Ph. D. attended during 2014/2016

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Abstract

The goal of the researches that make up the present study was to explore the potential of *C. cardunculus* L. to be exploited for some new uses.

Although the globe artichoke has deep roots in the culinary traditions of the Mediterranean countries, it requires nowadays innovations that remove the difficulties of its preparation and make it easier and faster to consume. The artichoke as a minimally processed and ready to use product addresses this need. The results presented in this thesis show that by choosing the right cultivar-packaging combination, it is possible to extend the shelf life of the product to at least 7 days from packaging.

The cultivated and wild cardoon produce large amounts of biomass, even under low-input conditions, thanks to their hardiness and the perfect match with Mediterranean climate. The data provided in this thesis confirm the cardoon as a possible source of renewable energy, both for its biomass yield and for biomethane yield of its silage. The volume of biomethane produced from the ensiled cardoon (between 200 and 250 Nm^3 per t DM) is comparable to that produced by the ryegrass silage, triticale and wheat. Silage of cultivated cardoon genotypes achieved volumes of biomethane (from 3,700 to 4,530 Nm^3 ha^{-1}) that are comparable to those obtained with maize grown under an intermediate input intensity regime.

Much of the biomass produced by globe artichoke plants remains in the field as crop residues, this biomass can be exploited for the production of bioethanol, considering its richness in complex carbohydrates (cellulose and

hemicellulose) and even in simple sugars (glucose and fructose). The enzymatic saccharification of non-pretreated biomass of residues of two globe artichoke cvs. ('Opera F1' and ‘Spinoso sardo') has given rise to a potential yield of 85-109 kg of EtOH per t DM and 1,440-1,520 kg of EtOH per ha.

Sommario

L’obiettivo delle ricerche esposte nel presente elaborato è stato esplorare la potenzialità di *C. cardunculus* L. di essere sfruttato per alcuni nuovi usi.
Anche se il carciofo ha profonde radici nelle tradizioni culinarie dei Paesi mediterranei, esso richiede oggigiorno delle innovazioni che rimuovano l’ostacolo rappresentato dalla sua preparazione e ne rendano più facile e immediato il consumo. Il carciofo come prodotto di IV gamma risponde a questa esigenza. I risultati illustrati nella presente tesi mostrano come attraverso la scelta del giusto binomio cultivar-packaging, si può prolungare la *shelf life* del prodotto ad almeno 7 giorni dal confezionamento.
Il cardo coltivato e quello selvatico producono una grande quantità di biomassa, anche se vengono loro forniti bassi input, grazie alla loro rusticità e alla perfetta sintonia con il clima mediterraneo. I dati forniti in questa tesi confermano il cardo come possibile fonte di energia rinnovabile, sia per la sua resa in biomassa, sia per resa in biometano del suo insilato. Il volume di biometano prodotto dall’unità di massa dell’insilato di cardo, tra 200 e 250 Nm^3 per t di sostanza secca (SS), è paragonabile a quello prodotto dagli insilati di loiessa, triticale e frumento. Gli insilati dei genotipi di cardo

coltivato hanno reso volumi di biometano per ettaro da 3700 a 4530 Nm^3, che sono paragonabili a quelli che si ottengono con mais con un medio livello di input.
La gran parte della biomassa prodotta da una pianta di carciofo rimane in campo come residuo colturale, questa biomassa potrebbe essere sfruttata per la produzione di etanolo, considerata la sua ricchezza in zuccheri complessi (cellulosa ed emicellulosa) e persino in zuccheri semplici (glucosio e fruttosio). La saccarificazione enzimatica della biomassa non pretrattata dei residui di carciofo di due cv. ('Spinoso sardo' e 'Opera F1') ha dato luogo a rese potenziali di 85-109 kg di etanolo per t SS e di 1440-1520 kg di etanolo per ettaro.

1 Historical outline of globe artichoke and cultivated cardoon and their cultivation in the world and in Italy

The English name «artichoke» derives from northern Italian «articiocco» (Skeat, 1887), which in turn derives, like the Spanish «alcachofa», the portuguese «alcachofra» and the Italian «carciofo», from the Arabic خرشوف (kharshuf) through the Hispanic Arabic «al-harsúf».

The generic name *Cynara* comes from the greek κύων (kyon - dog) (Quattrocchi, 1999) and probably from its diminutive κυνάριον (kynarion), which means little dog, puppy (Liddell and Scott, 1996). The reference to the dog has a negative connotation, because of the objectionable thorns, which are linkened to dog's teeth (Small, 2009). Pedanius Dioscorides, who was a physician, pharmacologist and botanist lived in the first century, in his "On Medical Material" spoke about κινάρα (kinara) and that word is translated in Bailly, Dictionnaire Grec Francais (1935) as a "kind of globe artichoke, cardoon". The name *Cynara* could be related to Κινάρος (Kinaros), an island of Aegean Sea, for a kind of globe artichoke native to that island (Quattrocchi, 1999).

The specific epithet *cardunculus* comes from Latin *carduus* with diminutive suffix -*unculus*, namely little cardoon.

Scolymus, that is the former specific epithet of globe artichoke and its current botanical variety, is the latinization of σκόλυμος (scolymos), which belongs to the semantic field of "thorn" (σκόλοψ - scolops). Hesiod, in his "Works and Days" (VIII-VII century BCE) used the word σκόλυμος to refer to *Scolymus hispanicus* L. (Liddell and Scott, 1996) or

to a kind of comestible cardoon or globe artichoke (Bailly, 1935).

From etymological overview emerges a certain vagueness in the ancient references, for this reason De Candolle (1890) suggested that cultivated globe artichoke was unknown in classical times. It has to be taken into account when reading, for instance, Pliny the Elder (23–79, in *Naturalis historia*) whose comments have been interpreted to indicate cultivated globe artichoke in south Italy and south Spain (Sonnante *et al*., 2007). Theophrastus (371–287 BCE) reported cultivation of globe artichoke in Sicily but not in Greece (Montelucci, 1962). Some roman mosaics conserved in the Bardo Museum in Tunis datable to the Imperial period (3rd century) show heads of globe artichokes (both spiny and spineless); Columella (1st century) in *De re rustica* talks about '*cinara*', defining it '*hispida*' (spiny). Foury (1989) deduced, based on Pliny and Columella writings, that the cultivation of globe artichoke started around the 1st century. Probably in the same period the domestication of globe artichoke was ongoing, but not yet accomplished (Sonnante *et al*., 2007).

We have little knowledge about globe artichoke in the middle Ages, contrary to the early modern period (sixteenth century) when it spread in Europe, as witnessed by paintings not only from Italy (**Figure 1.1**), but also from Flanders (**Figure 1.2**) and Bohemia (**Figure 1.3**).

Figure 1.1. Vincenzo Campi, “Fruttivendola” (1580). Pinacoteca di Brera, Milano.

Figure 1.2. Joachim Beuckelaer, “The Four Elements: Earth” (1569). National Gallery, London.

Figure 1.3. Giuseppe Arcimboldo, "Vertumnus" (1591). Skokloster Castle, Stockholm.

The most recent statistics about globe artichoke in the world show that the most important producer country is Italy (**Figure 1.4** and **Figure 1.5**) with more than 45,000 ha and ~440,000 t year^{-1}. Italy alone accounts for more than one-third of the world production (FAOSTAT, 2016). Traditionally globe artichoke is cultivated in Mediterranean countries, where is more than 70% of the world's globe artichoke growing areas (**Figure 1.4**), with nearly three-quarters share of the output (**Figure 1.5**), but other countries – especially Peru, Argentina, China and USA – are beginning to produce significantly (FAOSTAT, 2016).

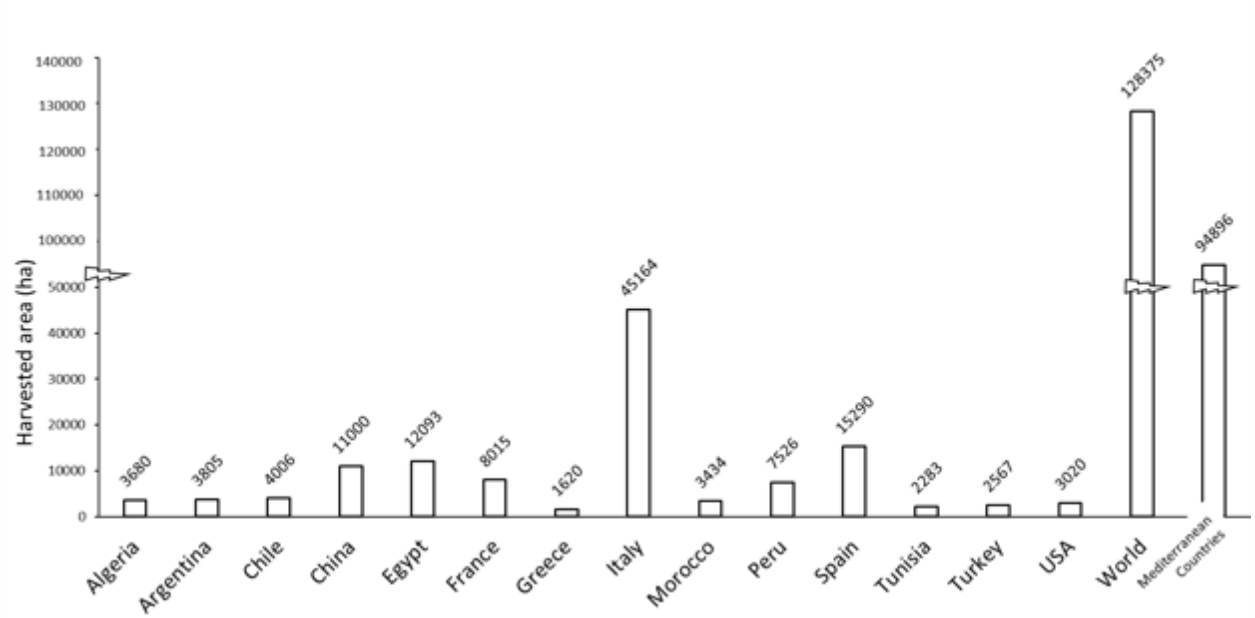

Figure 1.4. Globe artichoke harvested areas in countries with at least 1,000 ha harvested. Considered period 2010-2012 (source: FAOSTAT, 2016).

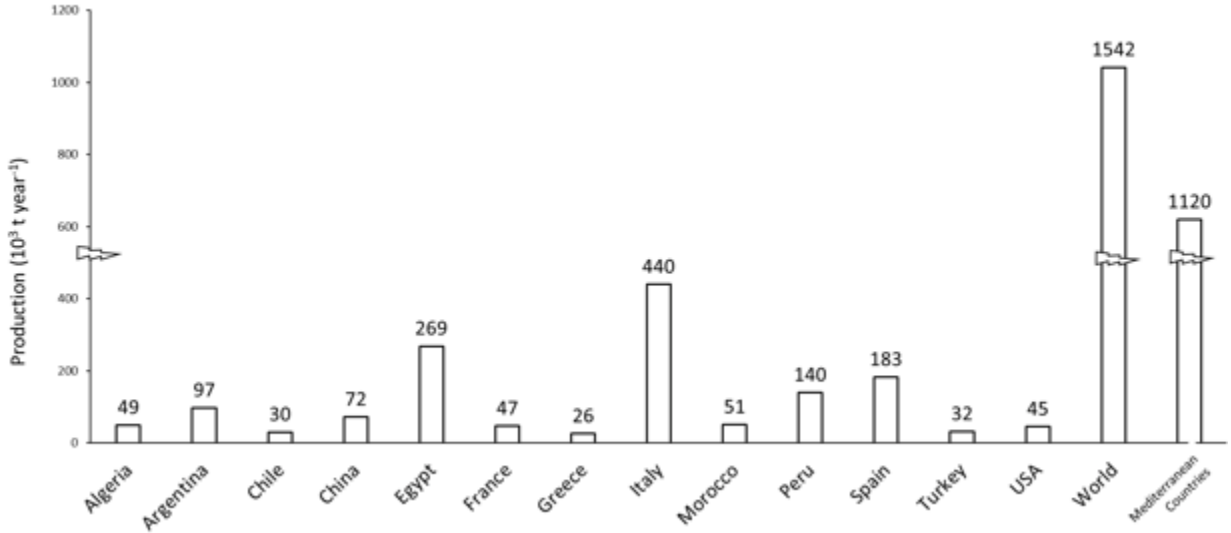

Figure 1.5. Globe artichoke annual production in countries with at least 1,000 ha harvested. Considered period 2010-2012 (source: FAOSTAT, 2016).

Regarding Italy, the most recent statistics – provided by ISTAT (2016) – show that Puglia has the largest harvested area, but Sicily is the most productive region (**Figure 1.6** and **Figure 1.7**). This is the sign that globe artichoke cultivation in Sicily is generally more rational and advanced than in Puglia. Sicily and Puglia together produce the ~63% of total globe artichoke in Italy (**Figure 1.7**).

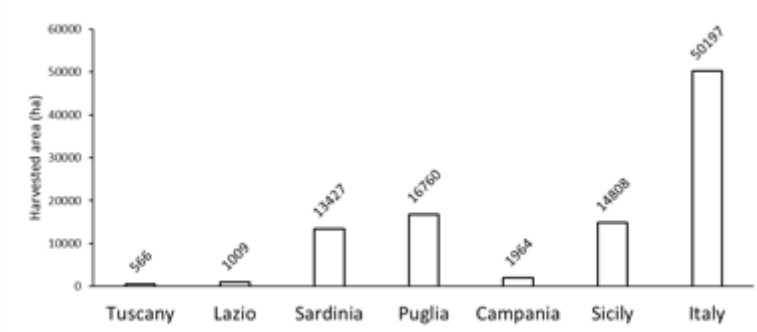

Figure 1.6. Globe artichoke harvested areas in regions with at least 500 ha harvested. Considered period 2009-2011 (source: ISTAT, 2016).

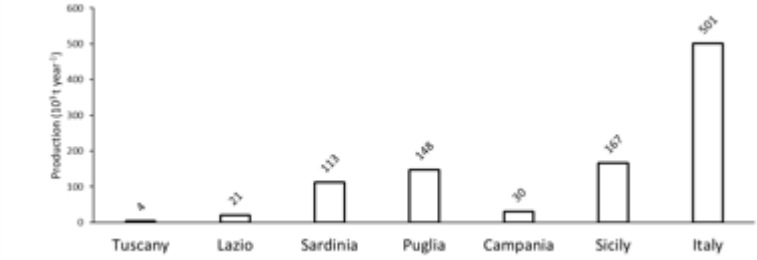

Figure 1.7. Globe artichoke production in regions with at least 500 ha harvested. Average of the period 2009-2011 (source: ISTAT, 2016).

Regarding Sicily, Caltanissetta province, which contains the Niscemi globe artichoke "district", is by far the most cultivated and productive province, with more than 40% of both cultivated area and production (**Figure 1.8** and **Figure 1.9**). Agrigento, which is the second globe artichoke producer province (thanks to the Licata globe artichoke "district"), has the same cultivated area of Chile but produces ten thousand tons per year more than this Country.
About cardoon, unfortunately there is not the same abundance of official statistics as for the globe artichoke. The areas devoted to cardoon cultivation (officially about 2–3000 ha, but this value is underestimated) are localized in Spain, Italy, France and Greece (Ierna and Mauromicale, 2010).

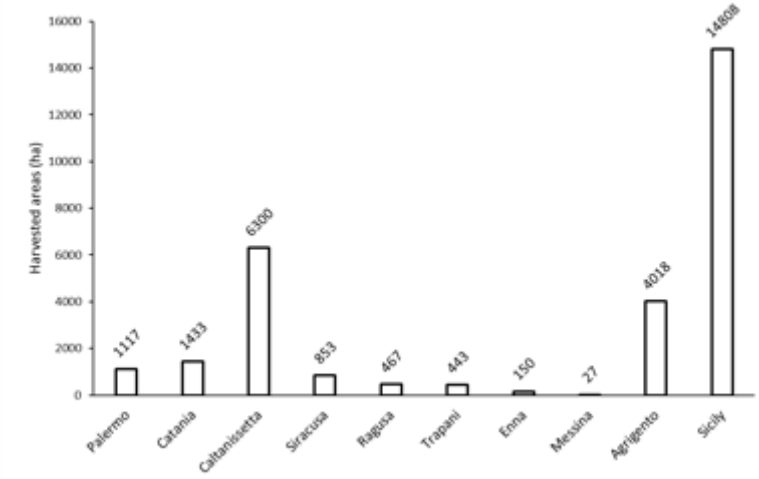

Figure 1.8. Globe artichoke harvested areas in Sicily provinces. Average of the period 2009-2011 (source: ISTAT, 2016).

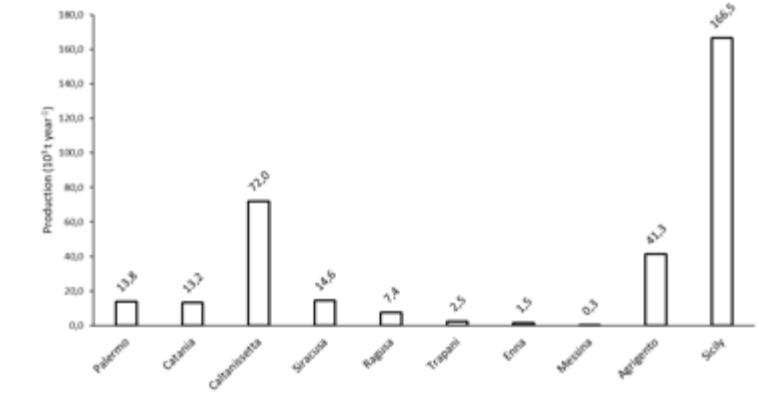

Figure 1.9. Globe artichoke productions in Sicily provinces. Considered period 2009-2011 (source: ISTAT, 2016).

2 *Cynara cardunculus* L.: botanical classification and description of the species

Cynara cardunculus L. is a species native to the Mediterranean basin, belonging to *Asteraceae* family, with diploid chromosome number (2n = 2x = 34). The taxonomy of the species belonging to genus *Cynara* is not so simple, because of the morphological similarity within *Cynareae* tribe, which requires the use of distinctive characteristics not always evident (Foury, 2004). The predominant allogamy of *C. cardunculus* led to formation of populations whose kinship are difficult to be understood. Studies about the isoenzymatic relationships (Rottemberg and Zohary, 1996), the intercross (Rottemberg *et al*., 1996), as well as on genetic and molecular relationships intra- and intervarietal (Lanteri *et al*., 2004a, b; Portis *et al*. , 2005a, b, c; Mauro *et al.*, 2009) lead to the conclusion that the cultivated botanical varieties of *C.*

cardunculus L., i.e. the globe artichoke [*C. cardunculus* L. var. *scolymus* (L.) Fiori] and cultivated cardoon (*C. cardunculus* L. var. *altilis* DC.), are phylogenetically very close to the wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori], which is probably the common ancestor of the first two. Presumably, from the latter species two divergent lines were implemented: one selected for the width of the leaves' midrib, leading to the cultivated cardoon, the other for the heads size, leading to the globe artichoke (Basnizki and Zohary, 1994; Lanteri *et al*., 2004b).

2.1 Globe artichoke [*C. cardunculus* L. var. *scolymus* (L.) Fiori]

Globe artichoke (**Figure 2.1**) is a perennial species that can be reproduced by achenes (improperly called "seeds") or propagated by "carducci" (side offshoots originating from underground buds) and "ovoli" (underground dried offshoots with apical and lateral buds). Plants from achenes have a primary taproot and numerous adventitious secondary roots, plants from "carducci" or "ovoli" have fibrous adventitious roots that enlarge over time (while the smallest disappear), losing absorption function in order to become storage organs and to provide support (Bianco, 1990). Their total depth is about 40 cm. The fleshy roots contain on average more than 20% inulin (on dry weight basis), 8 - 9% sucrose, 1.5% of reducing sugars and traces of starch. While the plant grows, a rhizomatous stem, commonly called "ceppaia", becomes more and more evident at the base of the main stem and on its surface several buds differentiate, giving rise to carducci. "Carducci" don't differentiate all at the same time, but in sequence because of apical dominance, so on the same

“ceppaia” buds at different age and physiological stage are usually present. Apical dominance intensity varies depending on the genotype (Mauromicale and Copani, 1990) and decreases when the main shoot differentiates the main head (La Malfa, 1976). The latest offshoots, which did not differentiate themselves into heads, dry up their aboveground fraction at the onset of summer drought, becoming “ovoli” (Jannaccone, 1967).

Figure 2.1. Biodiversity in globe artichoke

During the vegetative phase, the caule is very shortened, so the plant has some whorls of leaves, clustered tightly at its base, giving it a typical "rosette" form. The apical bud of each shoot becomes a flower stem, which has on its top the main inflorescence. The flower stem, variable in length depending on the variety, on the time of year and on the hormone treatments possibly provided, is cylindrical, ashy green colored, longitudinally ribbed, erect and has ramifications variable in number. It shows trichomes and alternate

lanceolate leaves. Stems of first, second, third (and so on) order also have a head at their distal end, whose size decreases as the order of ramification increases.
Leaves' size, shape and number are variable depending on the cultivar. They are ashy green colored on the lower side, have thick midrib and are typically fewer in the earliest cvs. (Foti and La Malfa, 1981).
Regarding the histological structure, the vegetative apex consists of *tunica* and *corpus*. In the *tunica*, two zones are recognizable, the apical one and lateral one, in which the beginnings of the leaves are recognizable. In the *corpus* are the procambium, the rib-meristem and flank-meristem, from which originate the beginnings of the leaves and the procambium. Overall the meristematic apex looks like a dome protected by young leaves. During the ontogenetic cycle of globe artichoke, the caulinar apex undergoes profound morphological and physiological changes, which lead to the differentiation and the emission of the inflorescence. Such phenomenon is mainly the result of the activity of caulinar apex meristem cells; these cells wrap parenchymal marrow first in several layers, then the layers become fewer up to shape a mantle-core structure; the floral primordia appear just beyond and they flatten their center, giving rise to surface of the thalamus: this represents the completion of the differentiation stage.
Macroscopically, the organogenesis of head is shown schematically through the following sequence of phenological stages (Foury, 1967):
R: indicates the caulinar apex transition from the vegetative phase to the reproductive one;

A: the head is perceptible to the touch but it is completely enveloped in leaf rosette that surrounds the caulinar structure;
B: the lengthening of the stem and the deployment of the leaves allow to see the head at the center of the leaf rosette;
C: the head is fully visible and the inflorescence reaches a length of 2-4 mm;
D: the flower stem is fully stretched and head has the optimum size to be harvested;
E: external bracts begin to diverge and the thalamus, which was concave, begins to flatten, while central flowers (florets) reach a length of about 2 cm;
F: the central bracts open, and in the center the first florets appear;
G: appearance of the flowers and anthesis of peripheral flowers.

2.2 Cultivated cardoon (*C. cardunculus* L. var. *altilis* DC.)

In a cultivated cardoon plant the underground part consists of the main taproot, of a variable number of secondary fibrous roots as well as of a rhizome, more or less expanded, containing buds, both single and gathered in groups.
The one-year roots keep the absorption function until the spring, when they swell and gradually assume the reserve and support functions, while a new adventitious root system takes place assuming the absorption function. Cardoon's root system is very developed, being able to reach a depth of more than 1 m, which allows the plant to explore a significant volume of soil. In plants older than a year, the underground stem becomes more and more evident, it is a rhizome commonly called also "ceppaia", which contains conducting

cells and reserve parenchyma. Several buds differentiate on rhizome (“ceppaia”) surface, giving rise to side shoots called “carducci”, which can grow up to form a new plant, thus expanding the rhizomatous fraction. In the epigeic part, cultivated cardoon shows a "rosette", since its stalk is very shortened (3-4 cm) and it has a high number of leaves (over 40), alternate and pinnate. Leaves can reach a length of more than 1 m (especially those basal), they are characterized by a well-developed petiole and a large midrib. The leaf lamina, crossed by numerous veins, could assume different shapes in different cultivars as well as within the same plant (heterophyllia). It is dark green colored or ashen on the top page, grayish on the bottom page, because of its thick hairiness. At flowering, the plant has one or more erect flower stems, of variable height (1.5 to 3 m approximately), each of which has a diameter of about 2-4 cm (**Figure 2.2**). Stems are longitudinally ribbed, tomentose and very branched, they also have little leaves alternate and lanceolate. Each branch has an inflorescence (head) at its distal end, which have a round or oval shape depending on cultivar. The plant has a high number of heads (10 to 30), the largest and earliest of which (main head) is on the top of the main stem; the heads of the next order (first, second, third and so on), progressively emitted, are gradually smaller. Each head has several hundreds of flowers (florets), which are hermaphrodite, tubular and fitted in a well-developed receptacle (thalamus). At the full anthesis, the florets have very long stigmata (~ 5-8 cm), usually blue-violet colored, although there are mutant genotypes having stigmata white or lilac colored. The fruit is a tetragon-shaped or flattened achene, dark-colored or

grayish, uniform or mottled; whose weight ranges between 20 and 40 mg.

Figure 2.2. The development in height of cultivated cardoon

Dissemination is favored by the calyx, metamorphosed into feathery organ (pappus). At physiological rippening of achenes, heads can reach a weight ranging from 10 to 120 g. From a biological standpoint, the cultivated cardoon is a perennial geophyte herbaceous species, whose field duration

is indefinite, thanks to the vitality of the rhizome. In the areas characterized by Mediterranean sub aride climate, where the crop express its full production potential, the crop cycle is autumn-winter-spring, with a vegetative stasis phase in the summer, more or less prolonged, while in middle latitudes the vegetative stasis occurs between spring and autumn. In Southern Italy, the cultivation cycle begins with the germination of achenes in the autumn, followed by a long vegetative phase, which lasts until the beginning of following spring. During this phase, the gradual transition to reproductive phase of the shoot apex occurs, and the first head starts to differentiate at late winter - early spring. The first head appears at first as a swelling in the center of the leaf rosette and becomes more and more evident because of the main flower stem elongation. At the same time, other heads progressively differentiate on the top of the branches of main floral stem. The anthesis starts in the late spring (late May - early June) and, of course, affects first the main head, then the other heads according to their order. At advanced flowering stage, each head presents several hundred florets at a different ripening stage. In every single head flowers' ripening starts from the most peripheral florets and proceeds centripetally until the center. In *C. cardunculus*, the flowering is dicogamous and, specifically, proterandrous. Indeed, the stigma becomes receptive from 4-5 to 8 days after anthesis, that is when the pollen, whose germination lasts 3-4 days, has already lost its vitality. Pollination is entomophilous and reproduction is mainly by cross-fertilization, because of the previously mentioned proterandry mechanism. However, a little amount of self-fertilization between different inflorescences of the same individual (geitonogamy) is

inevitable, due to their progressive ripening. The achenes' ripening take place starting from 50-60 days after anthesis and is accompanied by the progressive desiccation of the aboveground biomass. When achenes reach ripeness, they start to be disseminated by wind (anemochorous dispersal), thanks to the disintegration of receptacle and the peculiar structure of the pappus.
Given the lack of dormancy mechanisms, except in some special case (temperatures higher than 29-30 °C accompanied by anoxia), the "seed" is readily germinable when favourable environmental conditions are met (sufficient soil moisture and temperature between 14 and 24 °C). The vegetative regrowth after the summer is assured by the underground rhizome buds, which have remained dormant during the hot and dry season.

2.3 Wild artichoke or wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori]

Wild cardoon is a typical geophyte species, perennial, capable of both sexual reproduction and agamic propagation. It has well-developed roots, with a taproot able to penetrate to a depth of about 2 m, which allows the plant to tolerate water deficits, and a variable number of secondary roots. It has a rhizome, more or less expanded, containing buds, both single and gathered in groups. The stem is robust, simple and striated, it can be hairy or hairless, but without thorns. The basal leaves, arranged as a large rosette, are deeply engraved, pinnate, long up to 35 cm, white and hairy on the bottom surface, and provided on the edge of long spines (1-3 cm). Stem's leaves are simple, alternate and spiny. The flowering axis is erect, branched, rugged, striated longitudinally and

provided with alternate leaves. The main stem has branches that at their distal ends have heads. The plant height is variable, from 40 cm up to 120-130 cm. The flowers (florets), hermaphrodite, tubular, characteristic of the Asteraceae, are grouped in an inflorescence (head). Florets at full ripening can be 8 cm long or more, they are violet colored, but there are mutant genotypes with white (**Figure 2.3**) or pink florets. The fruit are tetragon-shaped or flattened achenes, dark grayish and mottled, combined with the calyx turned into pappus, which promote the anemochorous dissemination.

Figure 2.3. Wild cardoon with white-colored florets

3 Genetic resources in globe artichoke and in cultivated cardoon

The globe artichoke germplasm currently available is grouped and classified according to different criteria, which are based on the harvest time and on the morpho-metric features of heads. Regarding the harvest time, usually autumn-harvested cvs. and spring-harvested cvs. are distinguished in two different groups. The cvs. belonging to the first group, called "early", provide an almost continuous production from autumn to spring (from October to April) and include “Violetto di Sicilia” and various similar varieties (Masedu, Molese etc .). Other early cvs. are “Spinoso Sardo”, “Violetto Spinoso di Palermo”, “Violetto di Provenza”, “Violet Margot” and “Blanca de Tudela”. The cvs. belonging to second group, called "late" or "spring", provide productions only between March and May, and include “Romanesco” types and “Violetto di Toscana”, widespread in Campania, Lazio and Tuscany. “Camus de Bretagne” and “Blanc Hyérois” are also included. On the other hand, from the standpoint of head characteristics, cvs. are usually grouped according to the color of the outer bracts, the size, the shape and the presence of thorns on bracts. Regarding the latter characteristic, varieties are divided into "spineless" (“Violetto di Sicilia”, “Violetto di Provenza”, “Romanesco”, “Violetto di Toscana”, etc.) and "thorny" (“Violetto Spinoso di Palermo”, “Spinoso Sardo”, “Spinoso di Albenga”, etc.) (Mauromicale, 1987). A first attempt to do a classification was carried out by Porceddu *et al*. (1976), who gathered the globe artichoke germplasm into four main groups (**Figure 3.1**): (i) "Thorny" (“Violetto Spinoso di Palermo”, “Spinoso Sardo”, “Spinoso di Albenga”, etc.), (ii) "Violetti" (“Violetto

di Toscana", "Violetto di Chioggia", "Nostrano", "Violetto di Pesaro", etc.), (iii) "Romaneschi" ("Campagnano", "Castellammare", "Tondo di Paestum", "Camard", "Blanc Hyérois", "Camus de Bretagne", etc.), (iv) "Catanesi" ("Violetto di Sicilia", "Violetto di Provenza", etc.).

Figure 3.1. The four main types of globe artichoke, grouped on the head shape basis. A= "Thorny"; B= "Violetti"; C= "Romaneschi"; D= "Catanesi"

This classification, with appropriate corrections, has been updated by molecular investigations, carried out by AFLP (amplified fragment length polymorphism) (Lanteri *et al.*,

2004b). This study has shown that the genetic variability observed within accessions belonging to same varietal type was higher than that found between different accessions of the same varietal type, confirming the multiclonal composition of many seed-propagated cvs. The artichoke genome was recently decoded (Scaglione *et al*., 2016). The assembly covers 725 of 1,084 Mb that constitute the genome of the species. The sequence codifies for about 27,000 genes. The understanding of the genome structure of the artichoke is crucial to identify the genetic basis of agronomic characters and the future application of selection programs. In Italy globe artichoke cultivation is characterized by the diffuse use of local populations, which are often marked by a multiclonal composition, with a very broad genetic base and also with a high degree of heterozygosity (this is the reason of the widespread recourse to the agamic propagation). Despite the availability of such a wide biodiversity, globe artichoke cultivation in Italy is still mostly based on large-scale cultivation of a few cvs. In recent years, however, a strong varietal innovation is occurring, thanks to the introduction, in some areas, of new agamic propagated cvs. like "Violetto di Provenza", "Spinoso Sardo", "Romanesco C3", "Terom" and "Tema 2000" or seed-propagated cvs. such as "Opal F1", "Opera F1""Concerto F1", "Madrigal F1", "Harmony F1," etc. The increase in biodiversity, indeed, is the best way to: (i) meet the market requirements; (ii) better satisfy the productive chains (fresh consumption and processing industry); (iii) give temporal continuity to product quality; (iv) improve farmers' incomes.

Regarding cultivated cardoon, there are not many cultivars on the market today. They appear in the catalogs of a few seed

companies and they are the result of selection for “carducci” production for human consumption. The objectives of such selection are the size of the plant, the color and the texture of the petioles and leaf lamina. These genotypes, unlike globe artichoke, are marked by a lower degree of heterozygosity (from which derives the possibility of gamic propagation), and a minor genetic variability (Portis *et al.*, 2005b). Commercial cultivated cardoon genotypes are obtained mostly through mass selection based on maternal parent only; this is the reason why within each cv. there is some genetic and phenotypic variation, unlike the globe artichoke F1 hybrids. Genetic improvement has until now slightly concerned this crop, especially regarding energy uses. Cultivated cardoon for energy purposes may be chosen among traditional genotypes characterized by large biomass production. This is the reason why specific programs of variety constitution are needed, in order to implement cardoon cvs. specifically suitable for biomass and achenes production. In Italy, the most common cultivars of cultivated cardoon are “Bianco avorio” (vigorous, spineless) and “Gobbo di Nizza”, both grown in Piedmont. “Bianco pieno migliorato”, “Pieno inerme” and “Gigante di Romagna” (synonym of “Gigante inerme a foglia intera”) are cultivated in Emilia Romagna. In Spain, unlike Italy, cultivars are distinguished based on the use, so there are cvs. for fresh consumption and others for the processing industry. Among the first there are “Verde de Tafalla”, “Blanco de Valencia”, “Lleno de España” and “Rojo de Corella”. Among the latter, the most popular are “Blanco de Peralta” and “Verde de Peralta”. In France, the most used cvs. are “Blanc améliorée” and “Rouge d'Alger”.

4 Congener species

According to Rottenberg and Zohary (2005), *Cynara* genus comprises the botanical varieties of *Cynara cardunculus* L., as well as other six (maybe seven) species, all native to the Mediterranean Basin. The other species of the genus belong to the Mediterranean native flora and, like the wild cardoon, are perennial herbaceous plants with bushy habitus, thorny and with diploid chromosome number 2n = 2x = 34 (at least in the accessions until today studied); some of these species have highly endemic distribution (Bazniski and Zohary, 1994).

According to Wiklund (1992), other species of the genus *Cynara* are:

- *Cynara humilis* L .;
- *C. cyrenaica* Maire & Weiller in Maire;
- *C. algarbiensis* Coss. Former Mariz;
- *C. cornigera* Lindley in Sibthorp & Lindley;
- *C. Baetica* (Spreng.) Pau;
- *C. syriaca* Boissier;
- *C. auranitica* Post in Post & Autran.

In the above list, *C. syriaca* and *C. auranitica*, which in other classifications are considered as synonymous, are regarded as different species. Furthermore *Cynara tournefortii* Boiss. & Reuter is not in this list, as Wiklund (1992) has treated it as a separate taxon.

5 Traditional uses

5.1 Globe artichoke

The main product from globe artichoke plant is the head, mostly used for fresh consumption and to a small extent

destined for processing industry. According to the EC Regulation No. 963/98, published in the Official Journal of the European Communities of May 8 1998, the globe artichokes for fresh consumption must be: intact, sound (not affected by rotting or deterioration), clean, fresh in appearance, practically free from pests, practically free from damage caused by pests, free of abnormal external moisture, free of any foreign smell and/or taste.

The stems must be cut off cleanly and must not be longer than 10 cm. Heads are classified in three classes: Extra, Class I and Class II. Moreover, they are further divided according to the following calibration classes:

- diameter of 13 cm and over,
- diameter from 11 cm up to but excluding 13 cm,
- diameter from 9 cm up to but excluding 11 cm,
- diameter from 7.5 cm up to but excluding 9 cm,
- diameter from 6 cm up to but excluding 7.5 cm.

There are two ways to market the fresh product: in the first one, the product is packed in bundles with some leaves and part of the flower stem; in the second one, the product is marketed in boxes with a scape 10 cm long and without leaves (Bianco, 1990). Globe artichoke is also marketed in a various types of processed products, like dehydrated, deep freezed, freeze-dryed, dehydro-freezed, natural brine, pre-cooked, in oil, creams, pickled, vegetable soups, minimally processed products.

All the transformation processes begin with the receptacle turning and the trimming of the upper part of the bracts, in order to obtain so-called "bottoms" or "hearts" of globe artichoke. For deep freezing, the heads are previously blanched for 4-10 minutes, depending on the size of the

pieces, in solution of 0.7% citric acid. The deep-freezing takes place at -35 -40 ° C; storing performs at -20 ° C. The globe artichokes preserved in oil are prepared using small heads, collected at the end of the season when prices are low. During storage, the globe artichokes in oil or in natural brine may show white inulin crystals (Le Roux, 1978; Di Venere *et al*., 2005). To avoid the appearance of such flaw, the "hearts" are immersed for 30 minutes in a 5% solution of citric acid at 60-80 ° C.

5.2 Cultivated cardoon

Cultivated cardoon features in many Italian dishes, but its use is not so common. Nevertheless, there are several cultivated cardoon cvs., often linked to the areas where they are mostly produced. The so-called "carducci" represent the edible product, which have thick and fleshy leaf veins. To this end, "carducci" undergo the "whitening" technique before their harvest. This technique is carried out during the autumn months (from September to November), and aims to promote the etiolation of leaf veins in order to make them tender and juicy. Traditionally, this technique consists in tying the leaf rosette in the upper third, then covering it with opaque sheets (plastic, paper etc.). When "carducci" are "ripened", they are gradually collected from November to April. The manual harvest is carried out by cutting the plant at the collar. The marketable yield, i.e. without the outer leaves and the upper blade, reaches 15-20 t ha^{-1}.

As previously mentioned the cultivated cardoon is consumed mainly fresh or after cooking, but it is also consumed preserved in oil; while the freezed product lose its characteristic taste and is therefore not recommended. Like

its closest relatives, the cultivated cardoon, is also used to obtain vegetable rennet.

6 Possible alternative uses

Today more than ever the need to exploit non-food crops matches with the convenience to enhance marginal lands, which are heterogeneous in terms of soil and climate. However, this combination requires the realization of the widest possible biological diversity of cultivated germplasm; this objective can be achieved only through the cultivation of "new" species, ecologically well adapted and able to take advantage of the native resources of a given agro-ecosystem. Furthermore, not only dedicated crops, but also crop residues could be exploited for non-food purposes. In this regard, *C. cardunculus* L. has been reported as one of the most interesting species in semiarid Mediterranean environment.

6.1 Minimally processed products

Nowadays, there is a continuous increase in the demand for fresh and ready-to-use products, such as minimally processed vegetables. Minimal processing operations can cause undesiderable changes in the sensorial, nutritional and health-promoting properties by loss of soluble compounds or the formation of unstable components (Shahidi, 1997). The main enzyme involved in the browning reaction is polyphenol oxidase (PPO; EC 1.14.18.1), which produces dark pigments (melanoidins), unacceptable in terms of sensorial and nutritional quality and safety, since they would provide an excellent substrate for microbial spoilage (Lattanzio *et al*., 1994; Barbagallo *et al*., 2009). Weight loss is another

phenomenon that negatively influences globe artichoke marketability. These negative effects have led, over the last 20 years, to the focus of food technologies for the shelf life extension of fresh-cut fruits and vegetables. Globe artichoke attracts great scientific interest because of its established nutritional and antioxidant properties (Lattanzio *et al*., 2009; Lombardo *et al*., 2012). The nutritional importance of globe artichoke is mainly due to its high polyphenol content (Pandino *et al*., 2012) which, at the same time, makes it very susceptible to browning. Most studies on minimally processed globe artichokes have been focused on efficient ways to reduce this reaction and the growth of microorganisms, as well as on the use of innovative packaging (Del Nobile *et al*., 2009; Amodio *et al*., 2011; Restuccia *et al*., 2014).

6.2 Cellulose production

The increasing demand of paper and the necessity to conserve the forests make some herbaceous species considerably interesting, especially those characterized by short cycle (annual or biannual) and high growth rate (Gominho *et al*., 2001). Among them, the most common are halfah grass (*Stipa tenacissima* L.), hemp (*Cannabis sativa* L.), cotton (*Gossypium* spp.), Kenaf (*Hibiscus cannabinus* L.), flax (*Linum usitatissimum* L.) and miscanthus (*Miscanthus* x *giganteus* Greef *et* Deu.), all species producing a large amount of dry biomass (Gominho *et al*., 2001).

Gominho *et al*. (2001) evaluated the anatomical, histological, chemical and technological characteristics of wild cardoon's floral stems, in order to verify the attitude to paper production. The results of this study have revealed a

homogeneous longitudinal structure of the floral stems, suitable for mechanical pretreatments aimed at improving the technological characteristics. The fibers consist of fairly long cells (~ 1.3 mm), longer than those found in *Eucalyptus* spp. (Miranda *et al*., 2001) and over 75% of fibers exceed 0.5 mm. In technological terms, the yield of cellulose pulp was 43.5% for the whole stems, while it was significantly higher in perimedullary region (47%). Finally, the paper obtained, regardless of the finishing process, has good characteristics of resistance.

6.3 Biomass energy production

Increased use of renewable energy, already in the near future, may offer significant opportunities for Europe to reduce greenhouse gas emissions and secure its energy supplies (Mauromicale *et al*., 2014). In the long term, bioenergy crops could play a key role in achieving these objectives, and among them, perennial crops could provide the greatest potential (Bentsen and Felby, 2012). This development will be driven by further productivity increases of agri-energy systems and the introduction of energy crops with higher yield (Mauro *et al*., 2015). The crops dedicated to the production of bioenergy differ from conventional food and fodder crops, as they are optimised to have high energy efficiency compared with a low environmental impact (European Environment Agency, 2006). Several scientific papers have revealed the possibility of using the biomass (leaves, stems, flower heads and achenes) of *Cynara cardunculus* L. as solid bio-fuel, through direct combustion or pyrolysis (Damartzis *et al*., 2011; Ierna *et al*., 2012; Karampinis *et al*., 2012). Wild and cultivated cardoon are

more suitable for this purpose than globe artichoke for their greater productivity and hardiness. The interesting feature of wild and cultivated cardoon is the high biomass production compared with a relatively small demand for energy inputs (Fernández *et al*., 2006; Ierna and Mauromicale, 2010). This feature is due to the hardiness and adaptability to the climatic conditions of southern Europe, which lead to good performances even in marginal land and in dry conditions, namely in environments less suitable for traditional cultivations. Thanks to the autumn-winter-spring cycle, plants can exploit the autumn-winter rainfall available in the Mediterranean environment (Ierna *et al*., 2012). Moreover, the oldest roots, which have reserve function, support the sprouting of the dormant buds after the summer dormancy. Being a perennial species, *C. cardunculus* L. is suitable to be grown in a short cycle (up to 3 years) or multi-year long cycle (over 10 years). In the first case, the culture can be included in a rotation plan with grasses or legumes; in the second case, the culture may be appropriately grown in abandoned marginal land, with soil and environmental benefits. In the Mediterranean environment, the annual biomass production amounted to 20-25 t DM ha^{-1} for cultivated cardoon, of 12-16 t DM ha^{-1} for wild cardoon, and 7-9 for the globe artichoke (Ierna *et al*., 2012). In long term trials (7 years) conducted in Sicily (Mauromicale *et al*., 2014; Mauro *et al.*, 2015), wild cardoon, although less productive than the cultivated one, showed greater stability in annual biomass production. Like all perennial biomass crops, cardoon shows low yields in the first year (about 60% of maximum yield) (Ierna and Mauromicale, 2010; Ierna *et al*., 2012), and reaches the maximum values at the 5th or 6th year of cultivation. In

following years cardoon, especially the cultivated one, undergoes a yield decline, which is around 8-10 t DM ha^{-1} $year^{-1}$ (Angelini *et al*., 2009).

6.4 Oil production

C. cardunculus L. is interesting also for the production of oil for human consumption and biodiesel, although grain and oil yield is not very high. Indeed 2 t ha^{-1} of grain are hardly reached, while their oil content is around 23-25% on weight basis (Maccarone *et al*., 1999; González *et al*., 2004; Fernández *et al*., 2005). In *C. cardunculus* L. oil, triacylglycerols are the predominant fraction, while phospholipids and glycolipids are lower. The good characteristics for human consumption are due to a high content of oleic and linoleic acid, in a balanced ratio, as well as to the low amount of free acids, peroxides, linolenic acid and saturated fatty acids and to the absence of erucic acid. The overall quality can be considered intermediate between the corn and sunflower oil. Biodiesel obtained from cardoon oil has good technological characteristics, among which are: high flash point (185-190 °C), high cetane index (~49) (Encinar *et al*., 2002).

6.5 Biomethane production

The biomethane production by dedicated crop, is mainly based on the anaerobic digestion of cereals silages (Berglund and Börjesson, 2006, Dressler *et al.*, 2012), among these, maize silage is the most utilized. Common wheat and triticale, as autumn and winter crops, are also used, while the ryegrass silage is used less frequently. The cultivation of the cardoon as a dedicated crop for the production of biomass

destined to biomethane production through anaerobic fermentation could be a low input alternative to cereals silage. According to latest laws, in Italy biomethane obtained from crop residues and non-food crops is more subsidized than that obtained from food crops (Ministerial Decree of December 5, 2013). Use of crop residues and biomasses from dedicated crops with low environmental impact, such as cardoon, improves environmental performances of energy production through anaerobic digestion.
Until now, suitability of *C. cardunculus* for biomethane production was evaluated on globe artichoke, both on waste resulting from industrial transformation (Ros *et al*., 2013; Fabbri *et al*., 2014) and on field crop residues (De Menna *et al.*, 2016). Cardoon was also evaluated for biomethane production from its stalks (Oliveira *et al.*, 2012) and from the whole plant in co-digestion with cattle manure (Kalamaras and Kotsopoulos, 2014).

6.6 Bioethanol production

C. cardunculus L. lignocellulosic biomass is a potential source of saccharides that can be converted into bioethanol. The bioethanol conversion is typically carried out through the production of hexose and pentose sugars from cellulose and hemicelluloses (Blanch and Wilke, 1983). Cellulose is an important structural component of the primary cell wall of higher plants, together with lignin and hemicelluloses. These three polymers are closely associated and together compose the ligno-cellulosic biomass. Lignin and the partially crystalline nature of cellulose fibers are the most important obstacles to deconstruct the lignocellulose matrix and depolymerise its cellulosic content (Coccia *et al*., 2014). For

this reason pretreatments, such as dilute acid hydrolysis (Esteghlalian *et al*., 1996; Chandel *et al*., 2012), represent unavoidable processes for the depolymerisation of cellulosic and hemicellulosic fraction into glucose, xylose and other sugars. Some studies have been carried out to evaluate the suitability of cultivated cardoon as a substrate for bioethanol production, finding yields of ethanol ranging from 13 to 27 g per 100 g of raw material (Cotana *et al*., 2015; Fernandes *et al*., 2015).

6.7 Use for animal feeding

The use of *C. cardunculus* L. as fodder is still not widespread, despite its good quality and good agronomical features. Some studies have already highlighted the nutritive values of green fodder and straw of cultivated cardoon, as well as digestibility of whole cardoon seeds in ruminants (Cajarville *et al*., 1999, 2000). Green fodder can be obtained as a by-product resulting from the removal of "carducci" (side offshoots originating from underground buds) from commercial globe artichoke crops (Marzi and Bianco, 1967), or as fresh biomass coming from dedicated cultivated cardoon crops (Dellacecca and De Palma, 1981). The latter could be interesting especially in the southern Mediterranean regions, not only for the high biomass yields (about 90 t ha^{-1} of fresh fodder), but also for its availability during the winter, when the growth rate of many common fodder species is low. The crop, furthermore, does not require high inputs and benefits for most of its biological cycle of autumn-winter rainfall. Recently a research team of University of Sassari stood out with a spin-off regarding the exploitation for small ruminants of whole seeds, oilseed cakes and seed extracts coming from cardoon.

Such products are rich in by-pass proteins with good aminoacidic prophile (Corriere della Sera, 2015).

6.8 Extraction of active compounds

Since from ancient times globe artichoke has been exploited for its healthy properties. Ancient Romans knew its digestive, diuretic and choleretic virtues. Galen (II century) was the first to talk about it and after him Strabo, Pliny, Varro and Columella. The empirical and popular medicine, over the centuries, has made extensive use of artichoke decoctions, infusions and juices. In 1840, in front of the National Academy of Medicine (Académie nationale de médecine), Guitteau spoke for the first time about "cynarin", the active substance that a hundred years later Panizzi would have isolated and purified. Only in 1929 globe artichoke officially entered in pharmacology, thanks to the French Leclerc and Brel. From then on, many scientific studies on globe artichoke not only have confirmed the healing properties already proved by experience, but also identified new ones (Oliaro, 1967).

6.8.1 Inulin

Globe artichoke, like other members of the Asteraceae family, synthesizes and accumulates inulin in its storage organs. Inulin belongs to the group of fructans, carbohydrates formed by the repetition of fructose units. In globe artichoke, inulin is particularly abundant in the roots and in especially in the receptacle (Lattanzio *et al*., 2002). Humans, because of the lacking in the small intestine of the necessary enzymes for the fructans hydrolysis, cannot digest inulin. Nevertheless it has properties which make it a functional compound of great

interest. Inulin is a prebiotic compound, it promotes in the colon the growth of bacteria belonging to *Bifidobacterium* genus, which may help impede the growth of harmful bacteria within the colon, stimulate the immune system, favor the absorption of calcium and magnesium and promote the synthesis of vitamins of the B group (Gibson *et al*., 1995; Roberfroid *et al*., 1998). Inulin also seems to be involved in the regulation of cholesterol and triglycerides in the blood (Delzenne *et al*., 2002).

6.8.2 Antioxidant compounds

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Globe artichoke leaf extracts may reduce oxidative stress induced in human neutrophils (Rondanelli *et al*., 2009). Cynarin, caffeic acid, chlorogenic acid and luteolin are the main antioxidant compounds and all belong to the category of the polyphenols. In globe artichoke, the phenolic component is present both in leaves and in heads (Gebhardt, 1998; Fritsche *et al*., 2002; Mulinacci *et al*., 2004; Lombardo *et al*., 2010; Pandino *et al*., 2011) and its content varies according to physiological age of tissues (Lattanzio and Morone, 1979).

6.9 Use of florets in the dairy industry

Coagulation of milk casein for cheese production may follow chemical or enzymatic way. The traditional enzymatic coagulation involves the use of animal rennet (extracted from abomasum of young ruminants). There are several plant extracts used as coagulant agents as an alternative to animal rennet. They have a deep-rooted tradition especially in countries where the use abomasum of ruminants is limited for

religious reasons (Judaism, Islam). In Mediterranean countries the most used vegetable rennet is undoubtedly the one extracted from the florets of *Cynara cardunculus* L. Especially in the Iberian Peninsula there are different traditional cheeses product with this rennet (Barbagallo *et al*., 2007), which received the protected designation of origin (DOP): Torta del Casar, Queso de la Serena, Serra da Estrela, Queijo de Evora, Queijo de Nisa, Queijo de Azeitão. Also in Italy there are some traditional cheeses obtained with vegetable rennet from *C. cardunculus* L., one of these is so called "Caciofiore", it is one of the most ancient and traditional cheeses of Central Italy and its long history is witnessed by Columella in its *De re rustica* (I sec. D. C.). Probably Caciofiore is the ancestor of Pecorino Romano.

6.10 Use as ornamental plant

The Asteraceae family includes many species of very common ornamental plants, such as ageratum, *Aster* spp., chrysanthemum, dahlia, marigold, zinnia, and gerbera. Since the prevalent use of the globe artichoke is for human consumption, most of the commercial varieties are classified in groups based on the head characteristics and the ripening period. However, the idea of globe artichoke as an ornamental plant is not new in some European countries, especially where it is little known and is therefore perceived as exotic plant, especially in English gardens (Cocker, 1967). In recent years attention to *C. cardunculus* as ornamental plant has been drawn, both as a garden plant, where it can be used as an ornamental hedge to cover walls or fences or to separate spaces, and as a fresh or dried "cut flower" (Lanteri *et al*., 2012).

EXPERIMENTAL PART

Part 1: Globe artichoke as minimally processed and ready-to-use product

Globe artichoke is one of the oldest cultivated vegetables in Mediterranean area and, therefore, it has deep roots in the culinary traditions of Mediterranean countries. Nevertheless the complexity of its preparation and its susceptibility to browning limit its consumption on a wider scale. Then again, consumers are increasingly looking for products that allow them to save time, like ready-to-use food. Therefore globe artichoke could be valorized by identifying the most suitable cvs. to obtain ready-to-use products and by implementing transformation processes, based on active compounds to delay browning and on packaging to reduce water loss. In a few words: prolonging the shelf life.
The results presented in this part of the work have already been published in the following two articles:

- Pandino, G., Barbagallo, R.N., Lombardo, S., Restuccia, C., Muratore, G., Licciardello, F., Mazzaglia, A., Ricceri, J., Pesce, G.R. & Mauromicale, G. (2017). Quality traits of ready-to-use globe artichoke slices as affected by genotype, harvest time and storage time. Part I: Biochemical and physical aspects. *LWT - Food Science and Technology***,** *76 A*, 181-189.
- Rizzo, V., Sapienza, G., Restuccia, C., Mauromicale, G., Lombardo, S., Pesce, G. R., Rapisarda, M., Perna, S., Rizzarelli P. & Muratore G. (2015). "Shelf life evaluation of fresh-cut globe artichoke packaged in a compostable biobased film. *Italian Journal of Food Science, Special Issue*, 7-12.

7. Shelf life of ready to use globe artichoke slices

7.1 Introduction

Two trials on minimally processed and ready-to-use globe artichoke are discussed. In both trials attention was given to ready-to-use globe artichoke slices to be used directly for salads or boiled or fried. This could represent a new product with an added value and with the potential to increase globe artichoke consumption over the Mediterranean Basin.

On the basis of previous researches, the aim of these two trials was to study the influence of genotype, harvest time, storage time, packaging and their interactions on the quality maintenance of ready-to-use globe artichoke slices by measuring their phytochemicals content, polyphenol oxidase activity, antioxidant activity, colour changes and weight losses.

7.2. Materials and methods

Two trials were carried out: in the first trial, (from now onward referred to as GT - genotype trial), three genotypes were studied, while in the second one (from now onward referred to as PT - packaging trial), two different packagings were compared.

7.2.1 Experimental fields, plant material and management practices

The GT, was carried out during the 2013-2014 growing season at the farm of the University of Catania, located in the Plain of Catania (Sicily, Italy). The field hosting the trial was characterized by a soil type classified as Vertic Xerochrepts (Soil Survey Staff, 1999). The mean 30-year maximum monthly temperature of the area ranges between 14.8 °C (January) and 30.6 °C (July) and minimum temperature between 7.8 °C (January) and 22.3 °C (August). The other experimental field,

related to the PT, was conducted during 2014–2015 growing season in a farm located in Cassibile area (Sicily, Italy) where the soil type is classified as Calcixerollic Xerochrepts (Soil Survey Staff, 1999). The monthly average maximum temperatures range from 15.7 °C (January) to 32.5 °C (July), while monthly average minimum temperatures from 8.1 °C (January) to 22.2 °C (February). In both locations the climate is semiarid-Mediterranean, with mild winters and hot, rainless summers.

In the GT, three globe artichoke genotypes were studied: 'Apollo', 'Exploter' and 'Spinoso di Palermo'. 'Apollo' is a Romanesco-type genotype, with spherical-shaped heads that are harvested from the end of February until May. 'Exploter' is characterized by deep violet bracts and oval shaped heads with harvest time in March-May. 'Spinoso di Palermo' is an early reflowering multiclone genotype widespread in Sicily, producing ovoidal, green heads with purple shades and yellow spines, with an extended production period: from November to April. Plants were planted in the form of either semi-dormant offshoots ('*ovoli'*) or seedling with 3-4 leaves.

In the PT the 'Spinoso sardo' varietal type was used. Like 'Spinoso di Palermo', it is a thorny, early maturing cultivar which is normally harvested between November and April. Plants were planted as semi-dormant offshoots ('*ovoli'*). Just in the GT, the plant material was arranged in a randomized block experimental design with four replicates, consisting of twenty plants per each plot. In both trials, plants were planted 0.8 m apart within the row and 1.2 m apart between rows, adopting a plant density of 1.0 plant m^{-2}. Crop management (fertilization, irrigation, weed and pest control) was performed according to the standard commercial practice. Giberellic acid was not supplied to the plants during the crop cycle.

7.2.2. Heads harvest, post-harvest treatments and sampling

In the GT, for each genotype and replicate about 100 heads were harvested at marketable stage in early March and early April 2014. The samples for the PT were harvested at marketable stage during March-April 2015 period and consisted of homogeneous lots of 50 heads. For both trials heads were prepared by removing the inedible parts (leaves, floral stem and outer bracts) and trimming (at about 2 cm) the heads tips. The heads so obtained were sliced by a manual slicing machine at 5 mm thickness. The slices were then immersed in sanitizing solution (0.23 g L^{-1} active chlorine) for 5 min, rinsed with tap water at 12 °C for 1 min and immersed for 5 min in a the antibrowning solution made of 0,5% citric acid and 2% ascorbic acid. The excess solution was then eliminated by using a manual centrifuge.

In the GT, twelve slices (10±1 g each) were packaged into PET trays (23×17.5×2 cm) using a semi-permeable film (SP/BY - System Packaging s.r.l., SR, Italy; oxygen transmission rate (OTR): 3700 cm^3 m^{-2} 24 h^{-1}). All samples were stored at 4±0.5°C and 90-95% RH and analyzed after 0 (production day), 4, 7 and 11 days of storage. At each storage time, physical, chemical and enzymatic determinations were performed.

In the PT, about ten slices were placed in PET trays and packed in ordinary atmosphere using a Cast Polypropylene film with OTR of 3000 cm^3 m^{-2} 24 h^{-1} (from this point forward referred to as SS_C) and a compostable BIObased film, with OTR 55 cm^3 m^{-2} 24h^{-1} (from this point forward referred to as SS_BIO) kindly provided respectively by Rotocalco Mediterranea (Siracusa, Italy) and InnoviaFilms (Novara, Italy). All samples were stored at 4±0.5°C and 90-95% RH and analyzed after 0 (production day), 5, 8 and 12 days of storage. At each storage time, physical and chemical determinations were performed.

7.2.3. Biochemical and physical parameters analysis

7.2.3.1. Soluble sugars and inulin.

Soluble sugars (glucose, fructose and sucrose) and inulin content were determined following the method of Lombardo *et al*., 2016. Briefly, 1 g of freeze–dried sample was added with 40 mL of boiling water and the pH was adjusted to 7.0 with 50 mM KOH. The solution was kept at 85±2°C for 15 min. After cooling at room temperature, the volume was made up to 100 mL with deionized water. One aliquot of this extract was used for the direct analysis of free glucose/fructose, another was incubated for 30 min at 40±2 °C with sucrose to determine the fructose from sucrose and a third aliquot was incubated for 60 min at 60±2 °C with fructanase for the determination of total fructose. Absorbance was measured at 340 nm using a Shimadzu 1601 UV-Visible spectrophotometer (Shimadzu Corp., Tokyo, Japan). All data presented were expressed as g kg^{-1} of dry weight (DW).

7.2.3.2. Ascorbic acid, total polyphenols and antioxidant activity.

In both the GT and the PT, the ascorbic acid determination was carried out as previously described by Giannakourou and Taoukis (2003). Each extract (20 μL) was analyzed using an Agilent series 1200 HPLC (Agilent Technologies, Palo Alto, CA) equipped with ChemStation software (B.03.01). Separations were achieved by a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm; 5.0-μm particle size), conditioned at 25 °C, with a 0.2-μm stainless steel in-line filter. The mobile phase was HPLC-grade water with metaphosphoric acid to pH 2.2 at a flow rate of 0.5 mL min^{-1}. Total run time was 15 min. Ascorbic acid was identified by comparing its retention time and UV spectral data to commercially available standard. For calibration, appropriate dilutions (10, 20, 30, 40 and

50 μg mL^{-1}) of a standard stock solution (100 μg mL^{-1}) were analyzed. Quantification was achieved at 245 nm by comparison of the sample peak areas with the calibration curve. The ascorbic acid content (AsA) was expressed as mg kg^{-1} of dry matter (DM).

Total polyphenol content (TPC) in the GT was determined using the following modified Folin-Ciocalteu method (Cicco, *et al.*, 2009): about 0.1 g of freeze-dried material was diluted in 1 mL ethanol 70% and stirred at room temperature for 1h, with shaking. The mixture was centrifuged at 5000 rpm for 5 min at 25°C; then, a suitably diluted aliquot was purificated with a C-18 end-capped cartridge Phnenomenex-Strata (Castel Maggiore, Bologna, Italy) in order to avoid interference by other reducing substances in the assay, and then mixed with Folin-Ciocalteu reagent at room temperature for 2 min. Sodium carbonate (5%, w/v) was added and the mixture was allowed to rest at 40°C for 20 min in thermostatic bath. In the PT, total polyphenol content was measured by Folin–Ciocalteu assay (Singleton and Rossi, 1965). In the GT the content was expressed as g of chlorogenic acid equivalent kg^{-1} of DW, while in the PT it was determined on the basis of a standard calibration curve generated with known concentrations of gallic acid and expressed as g of gallic acid equivalent kg^{-1} of DW. In both trials the absorbance was read at 760 nm.

In the GT the antioxidant activity of the extracts was also evaluated as percentage inhibition of DPPH radical (Brand-Williams *et al.*, 1995). An aliquot (0.1 mL) of each extract used for total polyphenol assay, was added to 3.9 mL of freshly prepared methanolic solution containing 0.24 g L^{-1} DPPH, and held in the dark for 30 min at room temperature. Then, the absorbance was measured at 515 nm in the spectrophotometer. The percentage inhibition of DPPH was calculated according to Brand-Williams *et al*. (1995).

7.2.3.3. Polyphenol oxidase (PPO) activity.

In the GT, the catecholase activity of PPO was determined spectrophotometrically using a modified version of the method proposed by Espín *et al.* (1997), since other tested methodologies did not give the same reproducibility with globe artichoke. Ten grams of fresh artichoke head homogenate were added to 20 mL cold acetone (-20°C) and continuously stirred for 10 min. The homogenate was filtered through Whatman No. 42 paper (Milan, Italy) under vacuum on Buchner funnel and the obtained acetonic powder, collected and suspended in 15 mL 0.1 M citrate buffer (pH 5.8, which corresponds to the average pH of globe artichoke heads), was kept overnight at 4°C, before being filtered again through Whatman No. 42 paper under vacuum on Buchner funnel. The clear solution was then ultrafiltered in a cell equipped with a 50 kDa membrane (Millipore 8050, Milan, Italy). The enzymatic activity was assayed spectrophotometrically at 505 nm using catechol as phenolic substrate. The standard reaction mixture contained 0.9 mL of 0.04 M phenolic substrate, 0.1 mL of 0.093 M MBTH (3-methyl-2-benzothiazolinone hydrazone) chromophore coupling agent in methanol, 0.05 mL of DMF (N,N-dimethylformamide), 1.5 mL of 0.05 M sodium acetate buffer at pH 7.0 and 0.5 mL of enzymatic extract. The reaction was stopped at different times with 0.5 mL of 0.9 M H_2SO_4. Blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. One unit of PPO activity was defined as the amount of enzyme which produces an increase in absorbance of 0.001 per min at 25± 0.5°C under the conditions described above.

7.2.4. Physical analysis

In both the GT and the PT, three colour measurements on different points of globe artichoke slices were performed using

a compact tristimulus chromameter (N-3000, Nippon Denshoku Ind. C. Ltd, Tokyo, Japan) with an 8 mm diameter viewing aperture and C illuminant (CIE, 2° observer). The equipment was calibrated with a reference white tile (x = 83.47; y = 84.43; z = 95.16). According to CIElab standard, L* (lightness), a* (from green to red) and b* (from blue to yellow) values were recorded. Then, the two polar coordinates chroma (or saturation, C*) and colour intensity (or hue angle) $h°$, were calculated as follows: $C^* = (a^{*2} + b^{*2})^{1/2}$; $h° = \tan^{-1}(b^*/a^*)$. In the PT, analysis of weight losses were performed, together with texture analysis, using a ZwickRoell z 0,5 (Zwick GmbH & Co. KG, Ulm, Germany), and respiration rate through a Dansensor A/S Checkpoint (Ringsted, Denmark).

7.2.5. Chemicals and standards

All the reagents and solvents for the chemical determinations were purchased from Sigma-Aldrich (Milan, Italy) and were of analytical or HPLC grade. Bi-distilled water was used throughout this analytical trial

7.2.6. Statistical analysis

In the GT all data were subjected to analysis of variance (ANOVA) as a factorial combination 'genotype (3) × harvest time (2) × storage time (4)'. Means were separated by a least significance difference (LSD) test, when the F-test was significant. Percent values were transformed to arcsin √x (Bliss transformation) prior to analysis and then subjected to ANOVA; untransformed data were reported and discussed.

7.3. Results and discussion of the GT

7.3.1. Biochemical parameters

7.3.1.1. Soluble sugars and inulin.

Amongst the factors under study, harvest time explained more than 50% of the total variation for fructose (68.4%) and glucose (52.3%) (**Table 7.1**). In particular, glucose, as well as the other carbohydrates, showed a significantly higher content in globe artichokes harvested in April compared to those collected in March (22.9 and 18.3 g kg^{-1} of DW, respectively) (**Table 7.2**). In agreement with our findings, high carbohydrate levels have previously been associated with increasing temperatures (Rosenfeld *et al.*, 1998). Furthermore, the more intense solar radiation might have contributed to increase photosynthesis and accumulation of carbohydrates. Besides harvest time, the sucrose and inulin content was also significantly affected by genotype ($P \leq 0.001$) (**Table 7.1**). The inulin content varied from 118.2 to 230.6 g kg^{-1} of DW in 'Exploter') and Spinoso di Palermo', respectively, and was in the range of previously reported values (Schütz *et al.*, 2006; Pandino *et al.*, 2011). A similar trend was found for sucrose, with 'Apollo' showing the highest content (32.2 g kg^{-1} of DW) and 'Exploter' the lowest (20.7 g kg^{-1} of DW) (**Table 7.2**). According to Vijn and Smeekens (1999), the prerequisite to start the fructooligosaccharides (FOS) accumulation is the availability of sucrose, specific substrate for the activity of the 1-fructosyl transferase (1-SST), key enzyme for FOS synthesis. This suggests that a higher sucrose level contributes to a genotype-specific inulin content, as observed in our study. The analysis of variance revealed a significant influence of the effect of storage time × harvest time interaction for all considered carbohydrates,except for glucose, which was significantly influenced ($P \leq 0.01$, **Table 7.1**) by the interaction genotype × storage time. Results highlight a marked glucose loss during the

first 4 days of storage for ‘Apollo’ (-29%) and ‘Spinoso di Palermo’ (-25%), whereas for ‘Exploter’ a slight increase was detected up to 7 days of storage (**Figure 7.1**). Leroy *et al*.(2009) reported that in globe artichoke storage time causes a decrease in inulin content and an average degree of polymerization, accompanied by an increase of free fructose and sucrose due to depolymerization of inulin. In this study, the inulin content decreased during storage in the products from both harvests (**Figure 7.2**). It is worth mentioning that in April, respect to March, a higher loss of inulin was recorded during the 11 days of storage at 4°C. The different behaviour could be attributed to the activity and/or accumulation of 1-fructan exohydrolase (1-FEH), responsible for the hydrolysis of inulin under refrigerated conditions. In a previous study, changes in the activity of 1-FEH in burdock root during storage at low temperature were observed (Imahori *et al*., 2010).

Table 7.1. Mean square per each source of variation (percentage of total) resulting from analysis of variance.

Source of variation	Degree of freedom	Biochemical parameter							
		TPC	**AA**	**GLU**	**FRU**	**SUC**	**INU**	**AsA**	**PPO**
Genotype (G)	2	12.5^{*}	6.8^{NS}	4.2^{NS}	2.7^{NS}	27.8^{***}	29.8^{***}	3.0^{***}	57.6^{***}
Harvest time (H)	1	32.5^{**}	37.4^{*}	52.3^{***}	68.4^{***}	27.5^{***}	31.7^{***}	44.3^{***}	0^{NS}
Storage time (S)	3	0.9^{NS}	22.1^{*}	25.1^{***}	4.5^{**}	2.6^{NS}	25.0^{***}	37.3^{***}	27.9^{***}
(H) x (G)	2	12.4^{*}	2.7^{NS}	1.2^{NS}	5.5^{NS}	7.7^{NS}	4.7^{NS}	12.4^{***}	1.7^{NS}
(G) x (S)	6	14.3^{***}	5.2^{NS}	8.0^{**}	3.2^{NS}	7.9^{NS}	1.9^{NS}	1.4^{NS}	11.6^{***}
(H) x (S)	3	8.5^{NS}	8.3^{NS}	0.8^{NS}	11.9^{***}	10.0^{**}	5.9^{***}	0^{NS}	0.2^{NS}
Total mean square		**342.0**	**571.3**	**480.2**	**1024.3**	**2252.8**	**214467.1**	**3.7**	**181.6**

Note: TPC: total polyphenols content; AA: antioxidant activity; GLU: glucose; FRU: fructose; SUC: sucrose; INU: inulin; AsA: ascorbic acid; PPO: polyphenol oxidase. ***, ** and * indicate significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, and NS, not significant.

Table 7.2. Biochemical parameters of globe artichoke slices as affected by main factors. Different letters within the same parameter and main factor show significant differences (*LSD test, P≤0.05*).

	Biochemical parameter							
	TPC (g kg^{-1} DW)	**AA** (DPPH % inhibition)	**GLU** (g kg^{-1} DW)	**FRU** (g kg^{-1} DW)	**SUC** (g kg^{-1} DW)	**INU** (g kg^{-1} DW)	**AsA** (g kg^{-1} DW)	**PPO** (U mmol min^{-1} g^{-1})
Genotype								
'Apollo'	22.9^{a}	72.3^{a}	21.7^{a}	16.1^{a}	32.2^{a}	224.3^{a}	0.6^{a}	6.7^{a}
'Exploter'	19.9^{b}	74.9^{a}	19.5^{a}	13.5^{a}	20.7^{b}	118.2^{b}	0.4^{c}	2.7^{c}
'Spinoso di Palermo'	22.6^{a}	72.1^{a}	20.4^{a}	15.1^{a}	30.8^{a}	230.6^{a}	0.6^{b}	5.6^{b}
Harvest time								
March 2014	23.3^{a}	71.0^{b}	18.3^{b}	11.1^{b}	24.3^{b}	153.4^{b}	0.4^{b}	5.0^{a}
April 2014	20.3^{b}	75.2^{a}	22.9^{a}	18.7^{a}	31.5^{a}	228.7^{a}	0.7^{a}	5.0^{a}
Storage time (days)								
0	22.2^{a}	76.6^{a}	25.0^{a}	13.7^{b}	29.7^{a}	266.8^{a}	1.0^{a}	2.5^{c}
4	21.4^{a}	75.1^{ab}	20.7^{b}	14.9^{b}	29.6^{a}	215.4^{b}	0.4^{b}	5.6^{b}
7	21.3^{a}	70.7^{b}	18.9^{bc}	13.4^{b}	25.1^{a}	173.0^{c}	0.3^{c}	6.2^{a}
11	22.3^{a}	70.1^{b}	17.8^{c}	17.7^{a}	27.1^{a}	109.0^{d}	0.3^{c}	5.7^{b}

Note: TPC: total polyphenols content; AA: antioxidant activity; GLU: glucose; FRU: fructose; SUC: sucrose; INU: inulin; AsA: ascorbic acid; PPO: polyphenol oxidase. ***, ** and * indicate significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, and NS, not significant

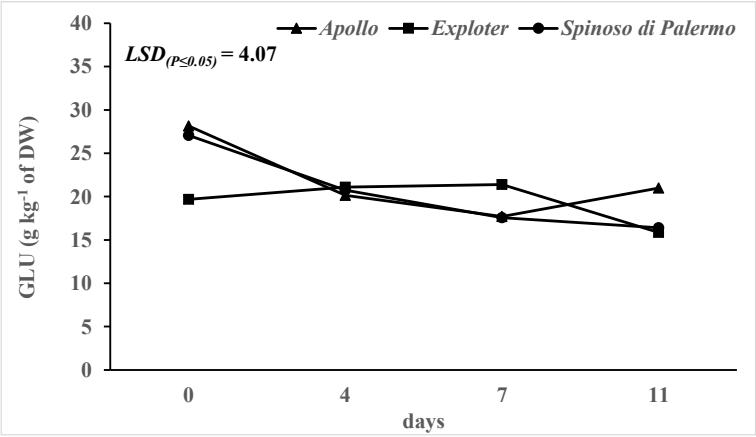

Figure 7.1. Glucose content (GLU), of globe artichoke slices as affected by 'genotype × storage time' interaction.

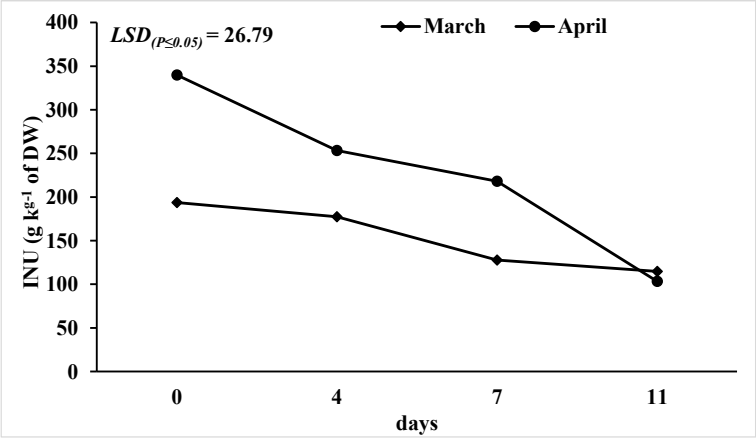

Figure 7.2. Inulin content (INU) of globe artichoke slices as affected by 'harvest time × storage time' interaction

Verhaest *et al*. (2007) reported that this enzyme is directly regulated by sucrose level in the plant. The higher sucrose content registered in April might explain these findings (**Table 7.2**).The fluctuant activity of 1-FEH affects sugars composition, since the depolymerization process produces free sugars, such as fructose. However, during storage an increase of the fructan 1-fructosyltransferase (1-FFT) activity was also observed through the synthesis of short-chain fructans (Van den Ende and Van Laere, 1996). In the present study, the highest level of fructose was found after 4 and 11 days of storage, respectively in April and March, while an opposite trend was recorded for the sucrose level (**Figure 7.3** and **Figure 7.4**).

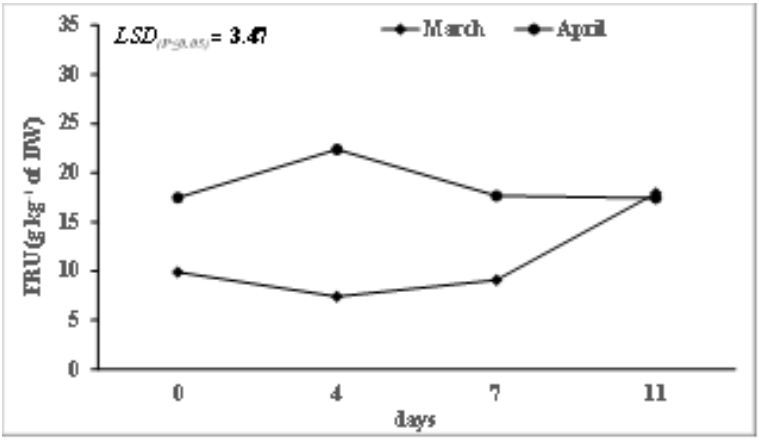

Figure 7.3. Fructose content (FRU) of globe artichoke slices as affected by 'harvest time × storage time' interaction.

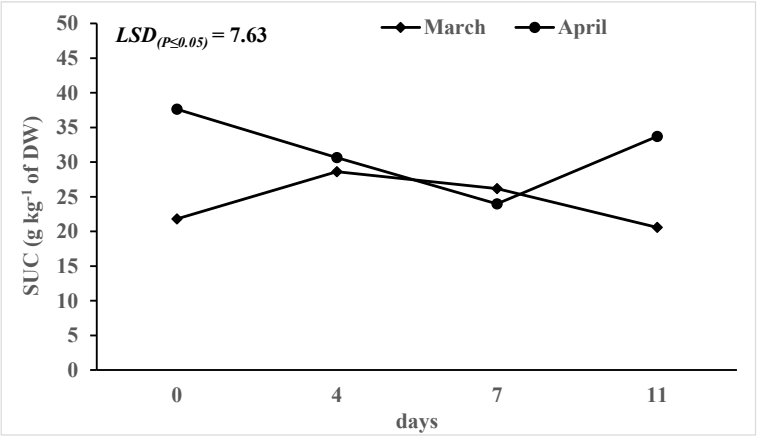

Figure 7.4. Sucrose content (SUC) of globe artichoke slices as affected by 'harvest time × storage time' interaction.

7.3.1.2. Ascorbic acid, total polyphenols and antioxidant activity.

ANOVA for the ascorbic acid (AsA) content revealed high statistical significance ($P \leq 0.001$) for the interaction genotype × harvest time (**Table 7.1**). 'Apollo' and 'Spinoso di Palermo' were characterized by the highest content of AsA when harvested in April respect to sample collected in March (on average, 0.3 *vs*. 0.9 g kg^{-1} of DW) (**Figure 7.5**). In relation to storage time, the ascorbic acid (AsA) content dropped by more than 70% during 11 days of storage (**Table 7.2**), in agreement with what reported by Kevers *et al*. (2007) for selected fruits and vegetables. The AsA content, which was increased with the dipping solution, is likely to undergo fast oxidation on the cut surface exposed to the headspace O_2.

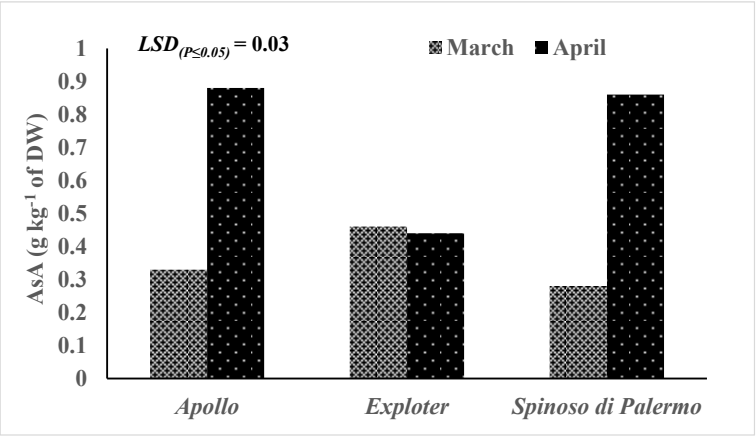

Figure 7.5. Ascorbic acid content (AsA) as affected by 'harvest time × genotype' interaction.

Similarly, the antioxidant activity (AA) declined significantly after 11 days of storage (**Table 7.2**), suggesting that the AsA content is the main compound affecting AA in globe artichoke slices. Our assumption was confirmed by the not significant variation found for TPC during storage. ANOVA revealed a significant influence of the genotype × storage time interaction for TPC ($P \leq 0.001$) (**Table 7.1**). The TPC showed the highest variation after 7 days of storage, ranging from 15.1 ('Exploter') to 26.0 g kg^{-1} of DW ('Spinoso di Palermo') (**Figure 7.6**). In particular, the TPC increased in 'Spinoso di Palermo' and decreased in 'Exploter' during 7 days of storage. The different behaviour of these genotypes is likely to be linked to their genetic background which is triggered by the stress caused by the processing and the storage conditions. Furthermore, a significant interaction genotype × storage time was observed by Muratore *et al*. (2015) in minimally processed globe artichoke heads. Conversely, Cefola *et al*. (2012) indicated not significant

influence of genotype × storage time on TPC. This variation could be due to the differences in postharvest handling and storage conditions, as reviewed by Soliva-Fortuny and Martín-Belloso (2003). Another possible explanation for the observed differences is that the TPC content in the present experiment was determined in fresh-cut globe artichoke after cutting and dipping, whereas the above authors measured the TPC content in the intact globe artichoke. The TPC was also affected by harvest time × genotype interaction (**Figure 3.7**). In particular, 'Exploter' heads harvested in March showed a higher TPC compared to those collected in April (23.3 *vs*. 16.6 g kg^{-1} of DW). These results are consistent with previous works, where the genotype × harvest time interaction significantly influenced the level of polyphenols in globe artichokes (Pandino *et al*., 2013; Lombardo *et al*., 2015; Pandino *et al*., 2015).

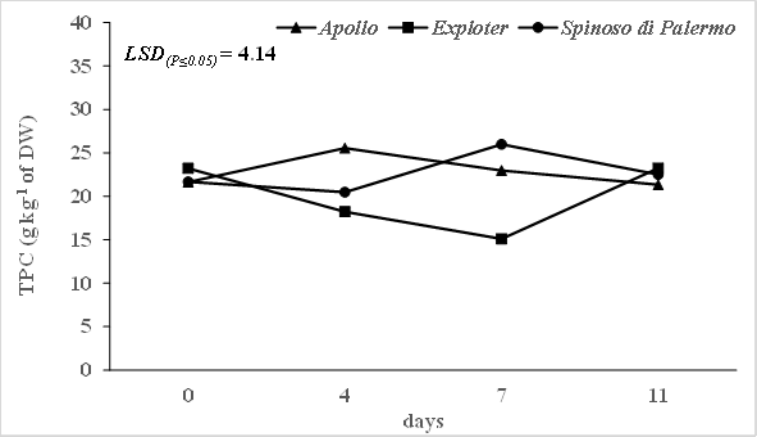

Figure 7.6. Total polyphenols content (TPC), of globe artichoke slices as affected by 'genotype × storage time' interaction.

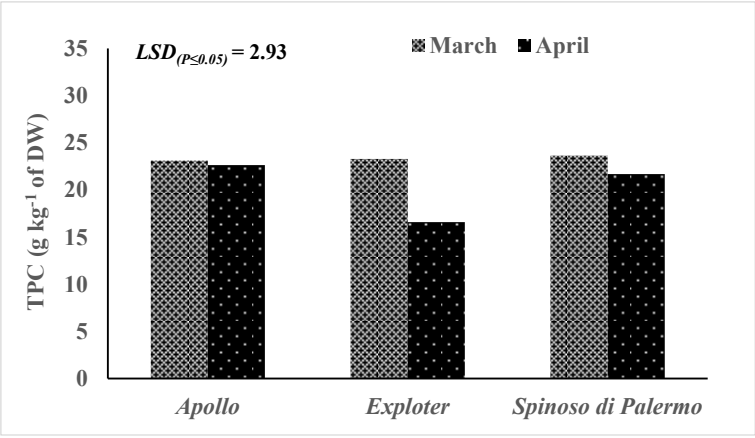

Figure 7.7. Total polyphenols content (TPC) as affected by 'harvest time × genotype' interaction.

7.3.1.3. Polyphenol oxidase (PPO) activity.

The significant genotype × storage time interaction confirmed the close interdependence between the suitability of globe artichoke genotypes for minimal processing and the shelf life of the commercial products. The different initial content of endogenous PPO thus represents a selective parameter which, in turn, is related with the proneness to undergo browning after minimal operations of shredding and cutting. 'Apollo' and 'Spinoso di Palermo' showed comparable PPO activities. In relation to changes of this enzyme during storage time (**Table 7.2**), its activity significantly increased during the first 7 days of storage, to decrease then, showing comparable values to those observed after 4 days. The decline is to be attributed to the consumption of the endogenous PPO involved with the phenolic component in the browning phenomena and loss of nutritional value (Leoni *et al.*, 1990), as well as to a molecular rearrangement of the phenolic compounds, specific substrates

for PPO activity. **Figure 7.8** shows that both 'Apollo' and 'Spinoso di Palermo' had a lower endogenous PPO concentration at processing time (2.3 and 2.2 U mmol min^{-1} g^{-1}, respectively) compared to the 'Exploter' (3.0 U mmol min^{-1} g^{-1}). In addition, 'Apollo' and 'Spinoso di Palermo' had a similar pattern, showing an increase in the PPO activity up to 7 days of storage: this trend is probably due to the activation of browning reactions catalyzed by specific phenolic substrates, available through the mediation of phenylalanine ammonia-lyase (PAL; EC 4.3.1.5).

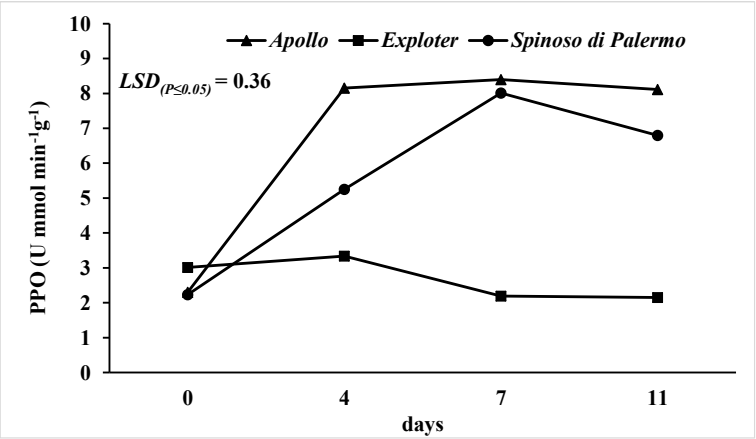

Figure 7.8 Polyphenol oxidase (PPO) as affected by 'genotype × storage time' interaction.

Previous papers (Lattanzio *et al*., 1989; Leoni *et al*., 1990) reported changes in phenolic compounds, PPO and PAL activities and the formation of iron/chlorogenic acid complexes in fresh marketable globe artichoke heads during refrigerated storage. This small activation of PPO could be induced by ageing phenomena, and/or by stress conditions during storage. In addition, the browning increase could be

explained by a greater availability of PPO following its release from the cell structures by other endogenous and degenerative enzymes, primarily polygalacturonase (EC 3.2.1.15), involved with pectin methyl esterase (EC 3.1.1.11) in cell wall solubilization (Barbagallo *et al.*, 2009). In both 'Apollo' and 'Spinoso di Palermo' the PPO activity declined up to 11 days of storage, while a different behavior was shown by 'Exploter' which, despite a higher initial PPO activity, showed an initial slight increase followed by a progressive decline until stabilization between 7 and 11 days of storage. This behaviour is explained by the unavailability at these stages of phenolic substrates to react, as evidenced by lower TPC values and by the higher levels of AA compared to the genotypes 'Apollo' and 'Spinoso di Palermo', although with a modest content of AsA.

7.3.2. Surface colour parameters

Storage time resulted the predominant factor influencing colour changes of ready-to-use globe artichoke slices, showing the highest percentage of total variation for all the measured colour parameters (L*, a*, b*) and for the calculated ones (C* and h°) with higher percentages for L* (53%), C* (40.7%) and b* (40.1%), respectively (**Table 7.3**). **Table 7.4** shows a gradual decrease of all measured and calculated colour parameters, with a trend to a "plateau" between 7 and 11 days of storage for the L*, b* and C* parameters, presumably due to the inhibition of the degenerative reactions catalysed by the PPO isoenzymes. Due to the loss of cell compartmentalization, fresh-cut globe artichoke is considered more susceptible to enzymatic reactions (e.g. oxidases and pectinases) respect to the whole vegetable (Barbagallo *et al.*, 2008). ANOVA highlighted that

the harvest time × genotype interaction was significant for all measured colour parameters, except for L*. The colour change from green to red tonality was evident in 'Apollo' which presented an average value of a* (0.5) tending to 0 in the April harvest, as well as in the 'Spinoso di Palermo', with a decrease of this colour parameter by about 15% (**Figure 7.9**). An opposite behaviour was shown by the genotype 'Exploter', probably because the higher temperatures and increased biochemical effect of solar radiation in April increased the synthesis of some antioxidant compounds which do not contribute to the total polyphenol content (TPC), most probably carotenoids (Ferracane *et al*., 2008). Relating to the change of colour from blue to yellow, all genotypes highlighted an increase of the b* values from the first harvest to April, though with greater variations in the genotype 'Exploter', followed by 'Spinoso di Palermo' and 'Apollo' (**Figure 7.10)**. A similar trend was observed for the polar coordinate C*, although with greater variations for both the genotypes 'Exploter' and 'Apollo' (**Figure 7.11**). *h*° value was affected by the interaction genotype × storage time (**Table 7.3**). 'Apollo' showed, at the production day, the lowest intensity of the colour perceived by the human eye (81.8 against 85.5 and 86.1 of 'Spinoso di Palermo' and 'Exploter', respectively), but after 4 storage days it showed the highest value (89.4) (**Figure 7.12**).

Table 7.3. Mean square per each source of variation (percentage of total) resulting from analysis of variance.

Source of variation	Degree of freedom	Colour parameter				
		L	**a**	**b**	**C***	**h°**
Genotype (G)	2	3.5^{***}	9.0^{***}	1.6^{**}	1.6^{**}	2.0^{**}
Storage time (S)	3	53.0^{***}	10.8^{***}	40.0^{***}	40.7^{***}	47.4^{***}
Harvest time (H)	1	32.5^{***}	0^{NS}	31.4^{***}	30.3^{***}	1.7^{*}
(G) x (S)	6	2.9^{NS}	4.7^{NS}	4.7^{NS}	4.8^{NS}	12.9^{***}
(H) x (G)	2	0.9^{NS}	30.4^{***}	12.5^{***}	13.1^{***}	17.4^{***}
(H) x (S)	3	2.7^{NS}	18.0^{***}	4.4^{NS}	4.3^{NS}	7.9^{NS}
Total mean square		**147.9**	**11.9**	**130.4**	**134.5**	**122.8**

Note: L*:lightness; a*: from green to red; b*: from blue to yellow; C*: chroma; h°: colour intensity.

Table 7.4. Physical parameters of globe artichoke slices as affected by main factors. Different letters within the same parameter and main factor show significant differences (*LSD test, P≤0.05*).

	Colour parameter				
	L	**a**	**b**	**C**	**h°**
Genotype					
'Apollo'	37.0[b]	0.2[c]	10.9[a]	11.0[a]	86.8[b]
'Exploter'	37.7[a]	0.4[b]	10.3[b]	10.4[b]	87.3[a]
'Spinoso di Palermo'	36.8[b]	0.6[a]	10.6[b]	10.6[b]	86.7[b]
Storage time (days)					
0	40.1[a]	0.8[a]	13.1[a]	13.2[a]	84.5[d]
4	37.2[b]	0.2[c]	10.2[b]	10.2[b]	88.0[b]
7	35.8[c]	0.2[c]	9.7[c]	9.7[c]	88.5[a]
11	35.6[c]	0.4[b]	9.4[c]	9.5[c]	86.8[c]
Harvest time					
March 2014	36.4[b]	0.4[a]	9.9[b]	9.9[b]	87.1[a]
April 2014	38.0[a]	0.4[a]	11.4[a]	11.0[a]	86.8[b]

Note: L*:lightness; a*: from green to red; b*: from blue to yellow; C*: chroma; h°: colour intensity.

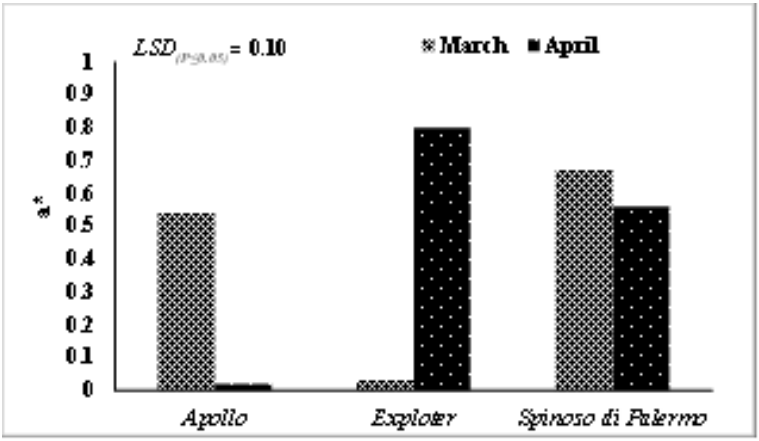

Figure 7.9. Colour variation from green to red (a*) as affected by 'harvest time × genotype' interaction.

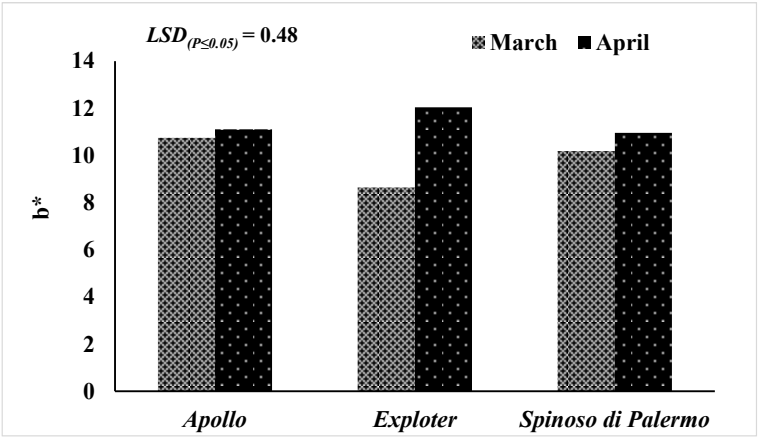

Figure 7.10. Colour variation from blue to yellow (b*) as affected by 'harvest time × genotype' interaction.

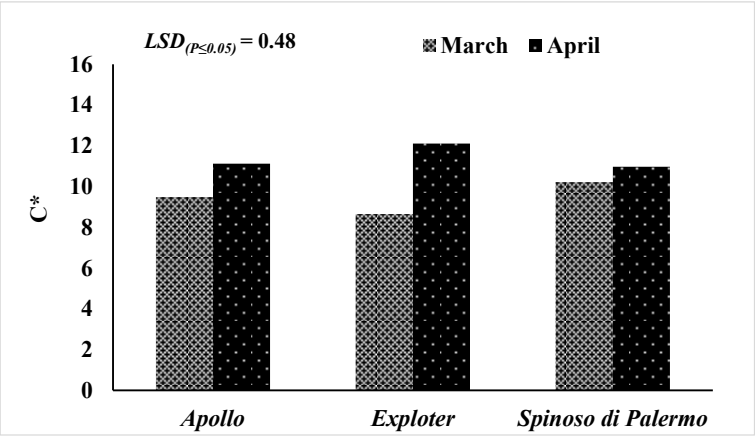

Figure 7.11. Chroma (C*) as affected by 'harvest time × genotype' interaction.

All the genotypes decrease their *h*° values from the day 7 to the day 11 of storage. This is presumably due to phenomena of tissue discolouration caused by an excessive presence of colourless intermediates (quinones). It is important to note that the *h*° parameter for 'Spinoso di Palermo' was almost unchanged during the first 4 storage days (**Figure 7.12**). The total range of variation of *h*° values in the tested genotypes wasl ow in the two harvest periods (**Figure 7.13**). The storage time × harvest time interaction was significant only for the a* parameter (Table 7.3). The latter was characterized by inverse trends in the early and late harvest: the samples from the heads harvested in March showed a progressive decrease in the a* values, which corresponds to the change from a green colour with reddish tones to a deeper green due to enzymatic browning reactions catalyzed by PPO.

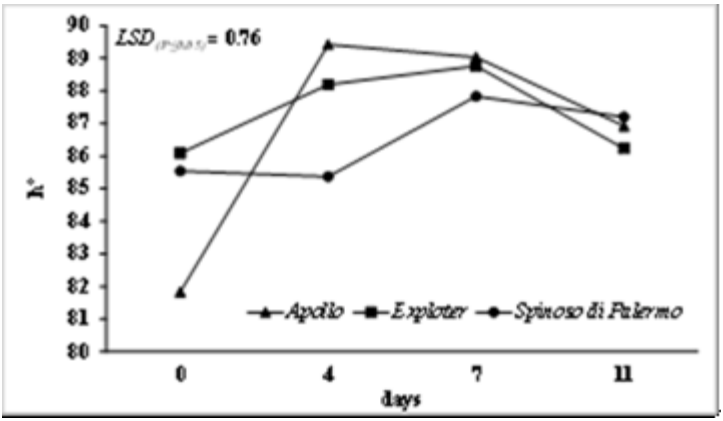

Figure 7.12. Colour intensity (h°) of globe artichoke slices as affected by 'genotype × storage time' interaction.

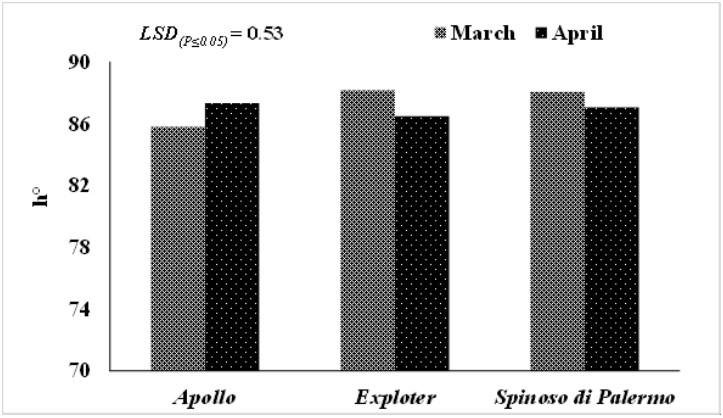

Figure 7.13. Colour intensity (h°) as affected by 'harvest time × genotype' interaction.

On the other hand, the combined action of the high April temperatures and of the increased photosynthetic activity determined a more effective antioxidant activity against the browning phenomena (**Figure 7.14**).

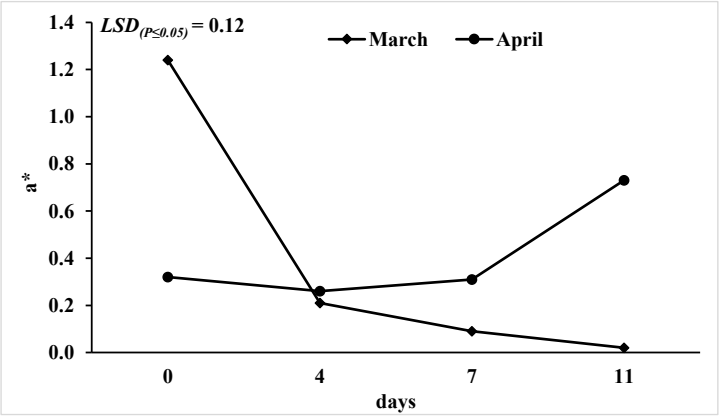

Figure 7.14. Colour variation from green to red (a*) of globe artichoke slices as affected by 'harvest time × storage time' interaction.

7.4. Result and discussion of the PT

As expected, weight loss in fresh-cut heads of cv. Spinoso sardo was higher in samples packed in the SS_BIO than in samples packed in SS_C bags (**Figure 7.15**). In fact, the compostable polymeric film has a higher water vapor transpiration rate (200 g m^{-2} d^{-1}, 25°C 75% RH). With reference to the texture (shear tests were performed on 6 different slices), the compostable SS_BIO showed the best performance even though with a high standard deviation among samples (**Figure 7.16**). As expected, in the package headspace of SS_C bags the oxygen concentration was lower than in the compostable SS_BIO bags. Whereas the carbon dioxide did not show significant differences between the packaging films (Figure 7.17)

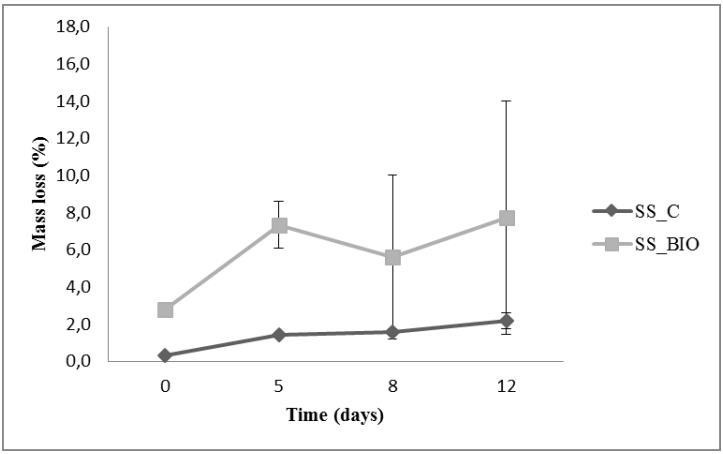

Figure 7.15. Effect of packaging film on the weight loss (%) of minimally processed globe artichoke heads during cold storage.

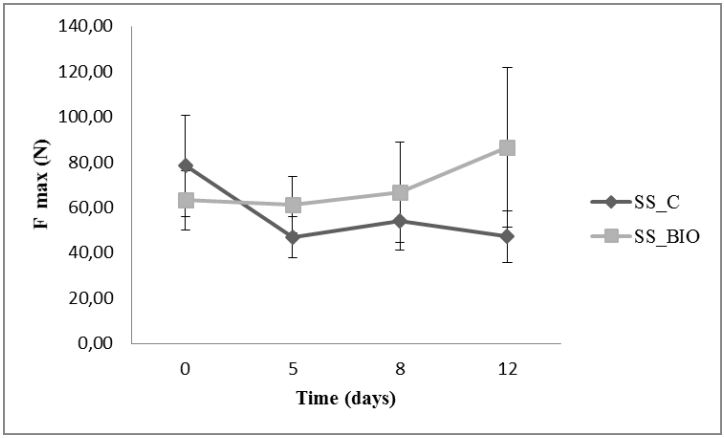

Figure 7.16. Effect of packaging film on the texture (N) of minimally processed globe artichoke heads during cold storage.

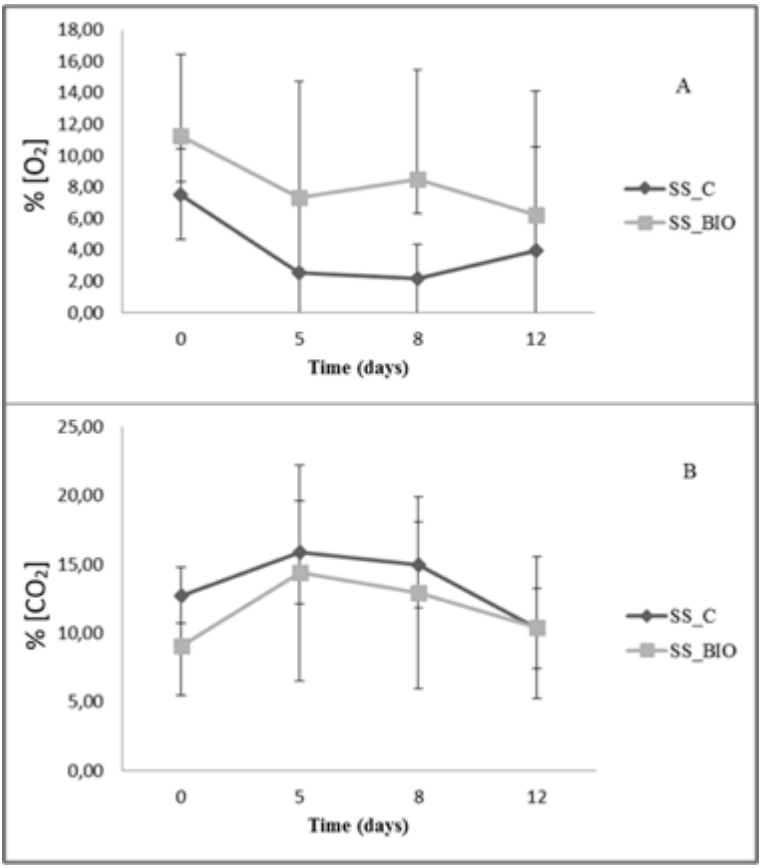

Figure 7.17. Respiration rate of minimally processed globe artichoke heads during cold storage, as effected by packaging film. Oxygen rate (A); Carbon dioxide rate (B).

At each sampling time, no differences were observed between the packaging films under study for both TPC and AsA (**Figure 7.18** and **Figure 7.19).** In particular, the TPC significantly decreased throughout the storage time up to 13.9 g kg^{-1} of DM for both packaging films, as observed previously by Ricci *et al*. (2013) due to an increase in

electrolytic leakage during cold storage. Similarly, AAC significantly decreased from the processing day up to 12 days of cold storage. This phenomenon may be attributable to AA conversion into dehydroascorbic acid (DHAA), which is less stable than AA (Davey *et al.*, 2000). The colorimetric analysis did not highlight great differences between the two packaging films even though, as reported in **Figure 7.20,** samples treated with SS_C showed higher L* values than those treated with SS_BIO.

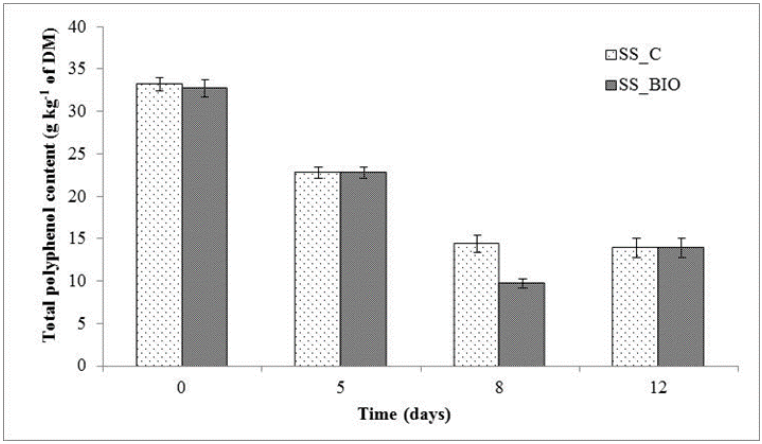

Figure 7.18. Effect of packaging film on the total polyphenols content of minimally processed globe artichoke heads during cold storage.

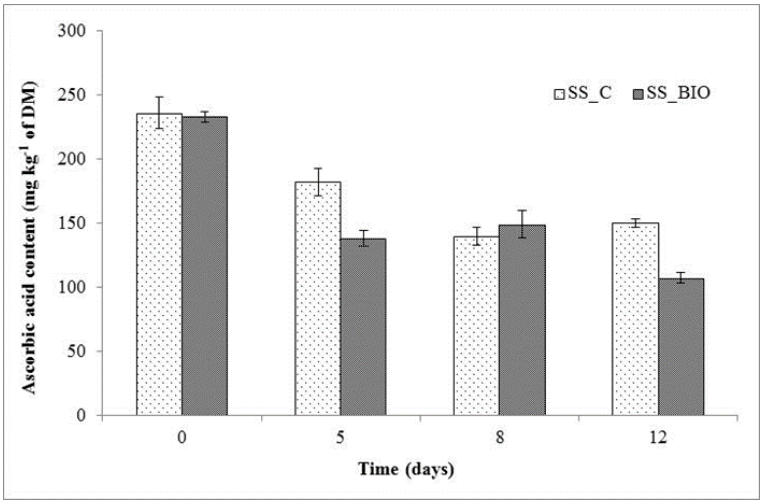

Figure 7.19. Effect of packaging film on the ascorbic acid content of minimally processed globe artichoke heads during cold storage.

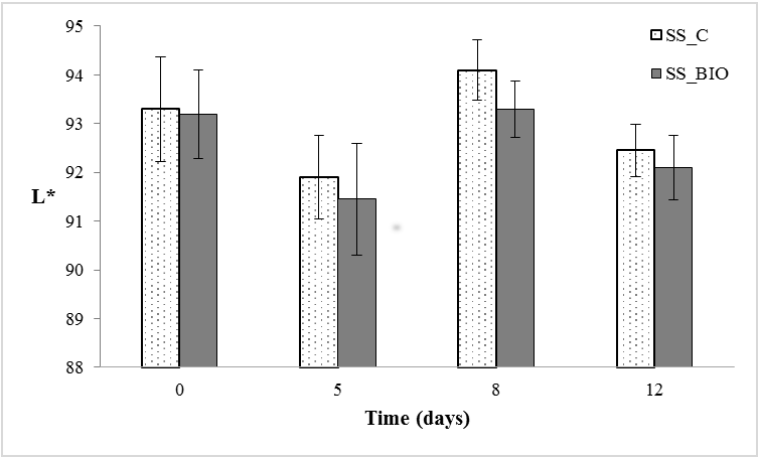

Figure 7.20. Effect of packaging film on the lightness degrees of minimally processed globe artichoke heads during cold storage period.

7.5. Conclusions

Results of the GT demonstrate that both biochemical and physical parameters of ready-to-use globe artichoke slices may be affected by several factors including genotype, harvest time and storage time. Harvest time was the predominant factor affecting the nutritional quality of ready-to-use globe artichoke slices, showing the highest percentage of total variation for six of the considered biochemical traits. On the contrary, colour parameters appeared to be strongly influenced by storage time. Overall, ready-to-use globe artichoke slices maintained high nutritional quality and colour parameters for at least 7 days of storage. Nevertheless, the fluctuation observed for both harvest time and storage time was found to be linked to the genotype, as demonstrated by the significant interactions among those factors. Therefore, food processors should be aware of the importance of genotype selection. Based on such consideration, 'Exploter' was the most suitable for processing as ready-to-use globe artichoke slices, especially in the late harvest, when it showed better colour parameters and lower content of TPC. Further studies are needed to clarify the contribution of other pre-harvest factors, such as agronomic practices and environmental conditions, on the quality of ready-to-use globe artichoke slices.

The PT was carried out by packaging in ordinary atmosphere the traditional globe artichoke cv. Spinoso sardo, with two different commercially available films: a Cast Polypropylene film and NatureFlex^{TM} compostable BIObased film. Results show better performances in the first one than in the latter, but the use of compostable BIObased film is interesting in order to reduce wastes and the environmental impact.

Part 2: *C. cardunculus* L. as energy crop

Both cultivated cardoon (*C. cardunculus* var. *altilis*) and wild cardoon (*C. cardunculus* var. *sylvestris*) have been proposed as possible bioenergy crops (Foti *et al*., 1999; Fernandez *et al.*, 2006; Gramellis *et al.*, 2008; Gominho *et al.*, 2011; Acquadro *et al.*, 2013; Mauromicale *et al*., 2014), since they produce a large quantity of biomass even when the plants are only provided with minimal inputs, which allows the crop to be targeted to land not usually used for cropping (Mauromicale *et al.*, 2014; Mauro *et al.*, 2015). Energy can be produced from the biomass either directly via its combustion (Foti *et al*., 1999; Fernandez *et al.*, 2006), while the oil accumulated in the achenes is suitable as a feedstock for biodiesel (Encinar *et al.*, 2002). The suitability of *C. cardunculus* biomass for biomethane generation has been thus far studied in the by-products of globe artichoke production (Ros *et al*., 2013; Fabbri *et al*., 2014; De Menna *et al.*, 2016), on cardoon stalks (Oliveira *et al.*, 2012) and on cardoon in mixtures with cattle manure (Kalamaras and Kotsopoulos, 2014). Alternatively, the high polysaccharide and low lignin content of cardoon biomass provides opportunities for ethanol production via fermentation (Gominho *et al.*, 2001; Ballesteros *et al.*, 2008; Cotana *et al.*, 2015; Fernandes *et al*., 2015).

8. The biomethane, silage and biomass yield obtainable from three accessions of *Cynara cardunculus*

8.1 Introduction

Under Italian law, biomethane produced from crop residues and non-food crops attracts a higher subsidy than that produced from food crops (Ministerial Decree of December 5, 2013). The use of crop residues or biomass obtained from a crop such as cardoon, which has a minimal environmental impact, improves environmental performances of energy production through anaerobic digestion (Duca *et al.*, 2015). The objectives of the present research were to assess the performance of two cultivated and one wild cardoon genotypes under low/zero inputs and to evaluate the yield of biomethane obtained by the anaerobic digestion of their biomass (leaves, stalks and inflorescence) ensiled at the beginning of capitulum and seed ripening.

The results presented in this part of the work will be published on Industrial Crops and Products, in the following article now under revision:

Pesce, G.R., Negri, M., Bacenetti, J. & Mauromicale, G. The biomethane, silage and biomass yield obtainable from three accessions of *Cynara cardunculus*.

8.2. Materials and methods

8.2.1 Local climate and soil

The experiment was carried out over three seasons, namely 2010-11 (S_1), 2011-12 (S_2) and 2012-13 (S_3). The

experimental site is located on the Catania plain in Sicily (37°26'N, 14°56'E), where the soil is classified as a Typic Xerofluvent (Soil Survey Staff, 1999) with a clay loam texture. The local climate is characterized by mild, wet winters and hot, dry summers. The ombrothermic diagram (**Figure 8.1**) shows the rainy and the dry periods, the latter are illustrated by dotted areas. According to Bagnouls-Gaussen (1957) classification, the local climate is thermomediterranean, with 5-6 months dry, from April to September. The mean seasonal rainfall over the period 1971–2000 was 446 mm, over 75% of which fell during the period October to March (CNMCA, 2009); the hottest month is August (26.6°C) and the coldest January (10.4°C). The aridity index, was calculated as the ratio between the annual rainfall and the annual reference evapo-transpiration (UNESCO 1979) estimated by using the FAO Penman-Monteith method (Allen *et al*., 1998).

8.2.2 Meteorological conditions during the trial period

Precipitation during the first two seasons (S_1 and S_2) was higher than during the third season (S_3); in both S_1 and S_2, it was above the long-term (1971-2000) mean (**Figure 8.1**, **Table 8.1**). The aridity index also underlined that S_3 was the driest of the three seasons, including a dry month during the normally rainy autumn/winter period (**Figure 8.1**). When all three seasons were taken together, February proved to be both the rainiest (132 mm) and the coldest (mean minimum/maximum air temperatures of 3.5°C/14.9°C) month. During the rainy period, October was the warmest (14.1°C/24.6°C) month. The hottest months were July and August (mean maximum air temperature of 33.6°C) (**Table 8.2**).

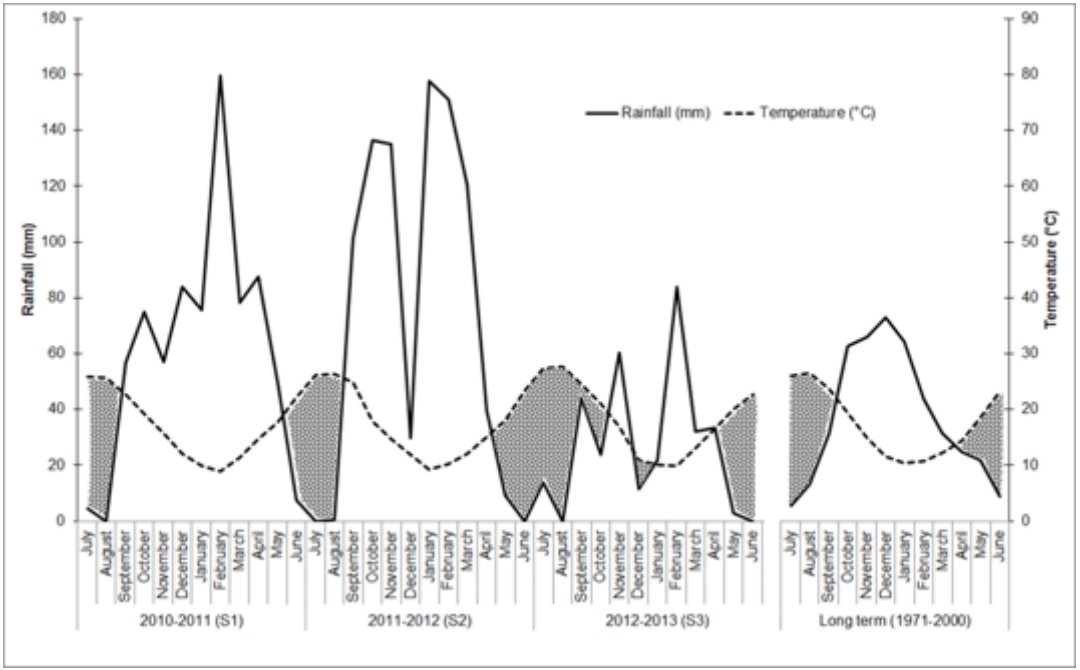

Figure 8.1. Ombrothermic diagram of 2010-2013 mean monthly temperature and monthly precipitation compared to the long term average (1971-2000).

Table 8.1. Comparison of monthly and seasonal rainfall (mm) between S_1, S_2, S_3 and long term period (1971-2000). The symbols + and - indicate values higher or lower than the long term value within each column.

	Month												**STR**	**SAI**
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun		
S_1	5 -	0 -	57 +	75 +	57 -	84 +	76 +	160 +	78 +	88 +	49 +	8 -	**736 +**	**0.49**
S_2	0 -	0 -	101 +	136 +	135 +	30 -	158 +	151 +	121 +	40 +	9 -	0 -	**881 +**	**0.55**
S_3	14 +	0 -	44 +	24 -	60 -	12 -	22 -	84 +	32 =	34 +	3 -	0 -	**328 -**	**0.20**
LT	6	13	31	63	66	73	64	44	32	25	22	9	**447**	

LT=long term; STR= seasonal total rainfall; SAI=seasonal aridity index

Table 8.2. Monthly averages of maximum and minimum temperatures (°C) during the three seasons.

		S1		S2		S3		Mean S1-S3	
		max	min	Max	min	max	min	max	min
	July	31,6	19,6	33,5	19,0	35,6	18,9	33,6	19,2
	August	31,6	19,7	33,6	19,5	35,7	19,4	33,6	19,5
	September	27,8	17,6	31,4	18,4	31,9	17,2	30,4	17,7
Rainy period	October	23,7	14,7	23,1	12,8	27,1	14,7	24,6	14,1
	November	21,1	10,5	19,3	10,2	22,3	10,8	20,9	10,5
	December	17,4	6,8	17,2	6,9	16,6	5,2	17,1	6,3
	January	15,9	4,3	14,7	4,1	15,7	4,4	15,4	4,3
	February	14,4	3,6	14,5	3,3	15,8	3,7	14,9	3,5
	March	17,0	6,2	19,0	5,8	18,5	7,7	18,2	6,6
	April	21,1	8,9	21,7	8,5	23,0	10,0	21,9	9,1
	May	23,6	12,0	25,9	10,1	27,0	13,0	25,5	11,7
	June	29,1	16,1	32,2	14,7	30,6	15,2	30,6	15,3

8.2.3 Experimental design and crop management

The experiment was set out in randomized blocks with four replications, each of which comprised 1,000 plants. The three genotypes comprised two cultivated cardoon lines, namely the University of Catania selection 'Altilis 41' (A41) and the cultivar 'Bianco avorio' (BA), while the wild cardoon population 'Sylvestris Marsala' (SM) was obtained from a native stand in Marsala (Western Sicily). Prior to planting, the field was ploughed to a depth of ~30cm, harrowed and a fertilizer dressing of 100 kg ha^{-1} N (as urea), 100 kg ha^{-1} P_2O_5 (as double perphosphate) and 80 kg ha^{-1} K_2O (as potassium sulphate) was given. Seedlings were transplanted in early November at the stage of fourth true leaf (about 40 days after germination) at a rate of 0.71 plants per m^2, using an inter- and intra-row spacing of 1.4 and 1.0 m, respectively. In S_2 and S_3, regrowth after the period of summer dormancy commenced in early September, when the first rains arrived. Just in S_1, two irrigations, each amounting to 20 mm (in April and May), were provided. Seedling establishment and early growth was supported by hand weeding. No pesticide or fungicide treatments were required.

8.2.4 Measurement of biomass and silage outcomes

In each of the three seasons, plants from the central area (measuring 14.1 m^2) of each plot were cut ~5 cm above ground level at phenological growth stage 8: capitulum and seed ripening (late June), when <20% of the heads were completely yellow (code 83 according to the BBCH scale by Archontoulis *et al*., 2010). The number of plants and the harvested biomass in the sample were recorded, as well as the weight of stalks, leaves and inflorescences material. The

tissue moisture content of each part was obtained by drying ~200 g samples at 105°C until a constant weight had been reached. For ensilage, ~30 kg of fresh material was first chopped, then packed into a sealed black polythene bag. More in details, the fresh biomass was chopped using a garden chipper (Model BIO 80 made by Caravaggi - Brescia) while the size of the particle ranges between 5 and 15 mm. The bags were stored for 90 days at room temperature (20±2°C) and then sampled for content analysis following Faithfull (2002). The following parameters were considered: dry matter (DM), pH, dry organic matter (DOM), ash, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), glucose, fructose, mannitol, ethanol, lactic acid, acetic acid, propionic acid and butyric acid.

8.2.5 Biogas and biomethane production

To assess the potential of the biomass to generate biomethane via anaerobic fermentation, three replicated samples of each of the three genotypes were prepared. The sample of silage were fermented without any pre-treatment and no additives were used. A set of unstirred lab scale fermenters was held at 40°C within a water bath. The inoculum was collected from various full-scale anaerobic digesters. In each fermenter, the inoculum/substrate ratio was 2:1 on a DOM basis: on average, each fermenter contained 1.4 kg inoculum and 100 g fresh silage biomass corresponding to about 37 g of dry silage biomass (Negri *et al*., 2014a). Fermentation continued until the quantity of gas produced by the inoculum + substrate was the same as by the inoculum alone. The volume of gas

generated was recorded on a daily basis. The chemical composition of the gas was monitored using a Combimass GA-m portable gas analyser (Binder, Ulm, Germany) equipped with an infrared dispersion cell to detect both biomethane and carbon dioxide.

8.2.6 Statistical analysis

All data were subjected to an analysis of variance (Snedecor and Cochran, 1989), and the means for each trait were separated by Fisher's least significance difference test, applying a threshold of 0.05. Values recorded as percentages were subjected to angular transformation prior to the analysis of variance.

8.3. Results and Discussion

8.3.1 Biomass yield

Averaged across all three seasons, the mean biomass accumulated by A41 was 19.1 t DM ha^{-1} per year, followed by BA (16.8 t DM) and SM (11.8 t DM) (**Figure 8.2**). This level of biomass yield agrees well with those experienced in similar trials carried out by Gherbin *et al*. (2001), Angelini *et al*. (2009), Ierna *et al*. (2012) and Mauromicale *et al*. (2014). The performance of the cultivated cardoon genotypes was equal or higher than that achieved by maize, wheat, ryegrass and triticale. Maize, a crop widely grown in Italy both as fodder and as feedstock for bioenergy via fermentation, can produce between 15.0 and 26.1 t DM ha^{-1} of biomass (González-García *et al*., 2013; Negri *et al*., 2014a; Bacenetti *et al*., 2014). The earliest maize classes, sowed late in spring following grain or fodder autumn winter crops, produce under irrigation a quantity of biomass comparable to that

produced by the cardoon genotypes described here, while late-maturing ones are much more productive. Wheat and triticale crops typically yield, respectively, 12.3 and 14.5 t DM ha^{-1}, while ryegrass yields in the range 8-15 t DM ha^{-1} (Bortolazzo *et al*., 2009; D'Imporzano *et al*., 2010; Soldano *et al*., 2013). All three genotypes accumulated more biomass during S_2 than during S_1, but less during S_3 than during S_2. The extent of the extra productivity during S_2 was most marked for A41, while the decline during S_3 was less marked for SM (**Figure 8.2**).

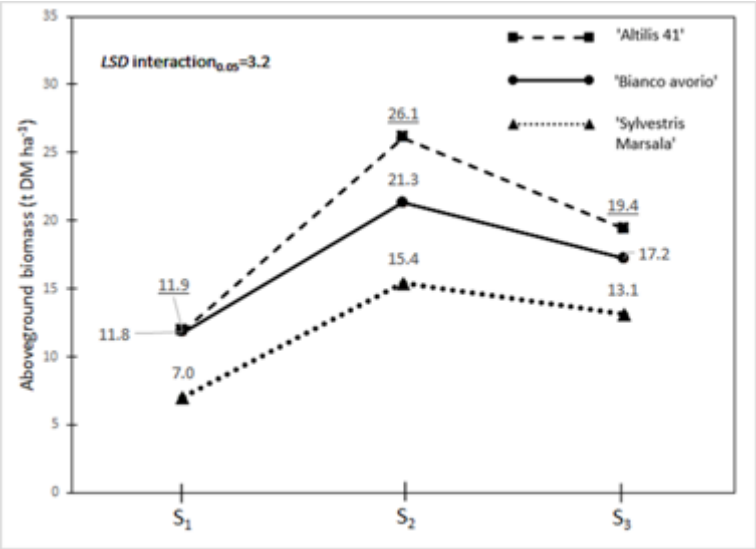

Figure 8.2. Effect of interaction "Genotype x Season" on cardoon aboveground biomass yield.

The loss of productivity during the third cropping season was unexpected, since in similar experiments carried out by others (Mauro *et al*., 2015, Mauromicale *et al*. 2014, Ierna and Mauromicale, 2010), the quantity of biomass produced in the

third year typically remains the same, if not more, than that produced during the second year (Angelini *et al*., 2009). A possible explanation for this unexpected result is that the rainfall experienced during S_3 was notably low (only 37% than that recorded in S_2) and the aridity index was very low (0.2, compared with 0.55 in S_2) (**Table 8.1**). By considering the biomass partitioning into the various plant parts, A41 produced the highest leaf, stalk and inflorescence material (7.2, 5.3 and 6.6 t DM ha^{-1} per year, respectively). The equivalent levels of performance for BA were 6.4, 4.0 and 6.4 t DM, respectively, and those for SM only 4.3 t, 2.8 t and 4.7 t DM, respectively (**Figure 8.3**). Leaf material accounted for 38% of the biomass accumulated by both A41 and BA, and for 36% by SM; stalk material for, respectively, 28%, 24% and 24%; and inflorescence material for, respectively 34%, 38% and 40% (Fig. 3). Between S_1 and S_2, the amount of leaf material increased by 192%, while the increase was less marked for both the stalk (44%) and inflorescence (79%). In contrast, during S_3 the proportion of leaf material decreased by 47% while the production of both stalk and inflorescence remained largely unchanged (**Figure 8.4**). During S_1, the DM was relatively equally distributed between leaf, stalk and inflorescence, while during S_2, 46% of the overall DM was composed by leaves, and during S_3 the relative contribution of the inflorescence was prominent (43%) (**Figure 8.4**).

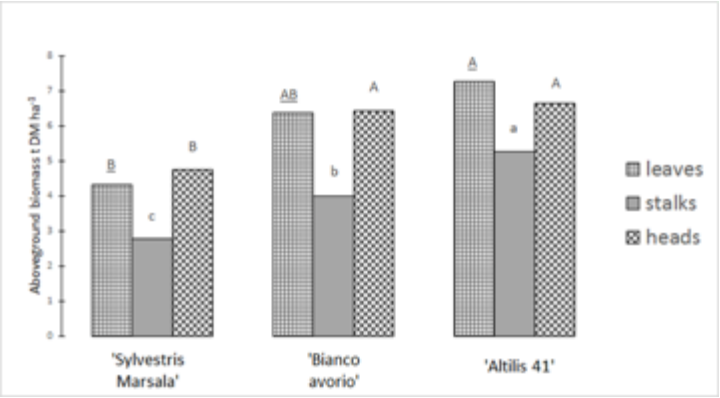

Figure 8.3. Mean values over three seasons of above-ground biomass yielded by each genotype, divided in leaves, stalks and heads. Different underlined capital letters indicate significance at *LSD* test (P≤0.05) within leaves. Different small letters indicate significance at *LSD* test (P≤0.05) within stalks. Different capital letters indicate significance at *LSD* test (P≤0.05) within heads.

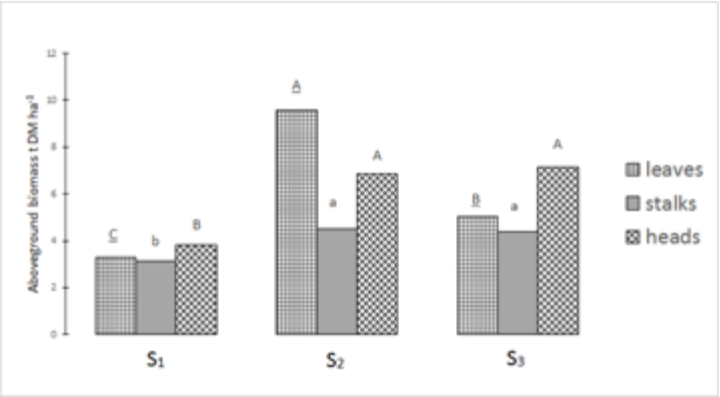

Figure 8.4. Mean values of above-ground biomass of the three genotypes yielded in each season, divided in leaves, stalks and heads. Different underlined capital letters indicate significance at *LSD* test (P≤0.05) within leaves. Different small letters indicate significance at *LSD* test (P≤0.05) within stalks. Different capital letters indicate significance at *LSD* test (P≤0.05) within heads.

This plasticity reflects the ability of cardoon to adapt to its environment: during a dry season it develops more reproductive material, while during a wetter one it develops more vegetative growth.

8.3.2 Silage characterization and biomethane production

Table 8.3 displays the outcome of the ensilage experiment. The DM content of the silage ranged from 32.8% in A41/S_2 to 37.1% in BA/S_3. These values indicate that the silage was made at the right time. The pH of the silage ranged from 3.3 (A41/S_2) to 4.1 (A41/S_3), this is the sign that the fermentation took place mainly by homolactic bacteria, which improve the fermentation process, accelerating the production of lactic acid resulting in rapid drop in pH. This confirms that the ensiling conditions were appropriate, and hence that there was only a modest loss of organic matter. The silage had a high ash content (10.8%-13.4%) and, consequently, a low DOM content (86.6%-89.2%). These values are very different from those prevailing in maize silage, which has a typical ash content of ~4% and a DOM of 96%, while both wheat and triticale silage contain ~10% ash and have a DOM of 90% (Negri *et al*., 2016). As a comparison, the ensiled whole plant maize contains at least 30% starch, while the ensiled maize ears can contain more than 60% starch (Negri *et al*., 2014b; Negri *et al*., 2016). The values of EE varied from 2.0% to 2.8%, a range which is similar to that seen in ensiled wheat, triticale and maize ears (Negri *et al*. 2014b; Negri *et al*. 2016). The evolution of biomethane over the 27 days fermentation of biomass harvested at the ends of S_2 and S_3 is reported in **Figure 8.5** and **Figure 8.6**, respectively. Both curves are well modelled by a quadratic relationship.

The fermentation of A41/S_2 biomass produced less biomethane than that produced by BA/S_2 biomass (195.1 *vs* 243.3 Nm^3 t^{-1} of DM silage), while the performance of SM/S_3 and BA/S_3 materials was effectively identical (~248 Nm^3 t^{-1} of DM silage), but superior to that of A41/S_3 (205.6 Nm^3 t^{-1} of DM silage). The poorer biomethane yield of A41 biomass could reflect the chemical constitution of the biomass, and specifically its higher proportion of stalks, which could be partially lignified, and the lower proportion of inflorescence material, where, moreover, the achenes are found.

Table 8.3 - Results of laboratory analysis for the different genotypes in S_2 and S_3. Different small letters within each variable indicate significance at LSD test ($P \leq 0.05$) in S_2. Different capital letters within each variable indicate significance at LSD test ($P \leq 0.05$) in S_3.

		S_2				S_3					
		'Bianco avorio'		'Altilis 41'		'Sylvestris Marsala'		'Bianco avorio'		'Altilis 41'	
DM	%	35,4	a	32,8	b	33,0	AB	37,1	A	34,6	B
pH	-	3,7	a	3,3	a	3,9	A	3,8	A	4,1	A
DOM	%	88,0	a	88,1	a	86,6	A	89,2	A	88,6	A
Ash[a]	%	12,0	a	11,9	a	13,4	A	10,8	A	11,4	A
CP[a]	%	12,8	a	14,2	a	13,9	A	13,4	A	14,6	A
EE[a]	%	2,8	a	2,5	a	2,3	A	2,4	A	2,0	A
NDF[a]	%	44,7	a	48,0	a	47,4	A	47,4	A	45,9	A
ADF[a]	%	33,2	a	28,1	b	33,3	A	32,4	A	37,4	A
Glucose[a]	%	0,3	a	0,3	a	0,2	A	0,3	A	0,2	A
Fructose[a]	%	0,5	a	0,5	a	0,3	B	0,5	A	0,6	A
Mannitol[a]	%	2,0	a	1,7	a	1,4	A	1,6	A	1,9	A
Ethanol[a]	%	0,5	a	0,5	a	0,5	A	0,4	A	0,4	A
Lactic acid[a]	%	0,9	a	1,3	a	1,4	A	0,8	A	1,3	A
Acetic acid[a]	%	1,4	a	1,4	a	1,0	B	1,9	A	1,9	A
Propionic acid[a]	%	0,2	a	0,3	a	0,2	A	0,5	A	0,4	A
Butyric acid[a]	%	0,3	a	0,2	a	0,2	A	0,1	A	0,2	A
C/N	-	27.0	a	22.5	a	22.7	A	24.1	A	22.0	A

[a] as fraction of dry matter
DM=dry matter; DOM=dry organic matter; CP=crude protein; EE=ether extract; NDF=neutral detergent fibre; ADF=acid detergent fibre

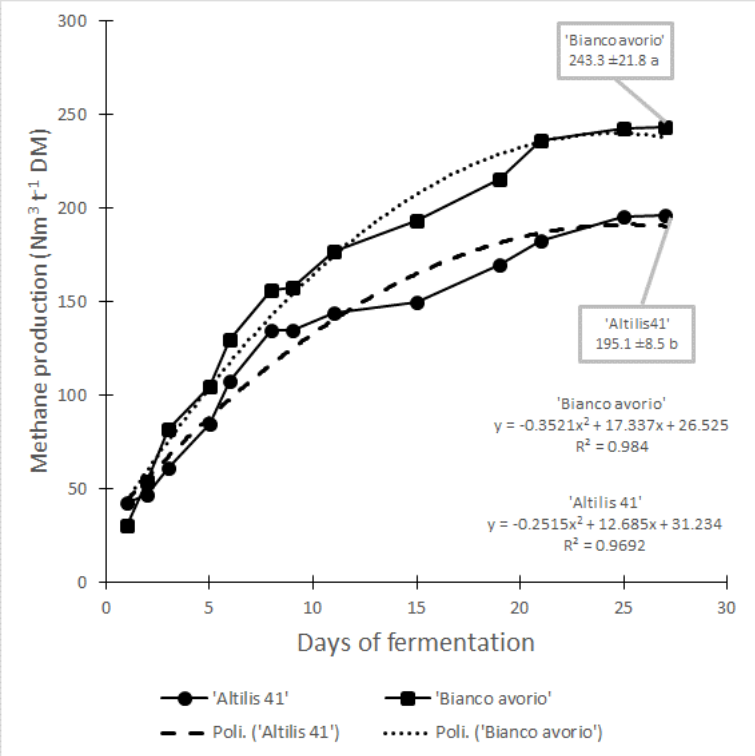

Figure 8.5. Cumulative curves of biomethane production along the fermentation period of two cultivated cardoon genotypes in S_2. Data reported in the rectangles are means (N=6) after 27 days of fermentation ± standard deviation. Different letters indicate significance at *LSD* test ($P \leq 0.05$).

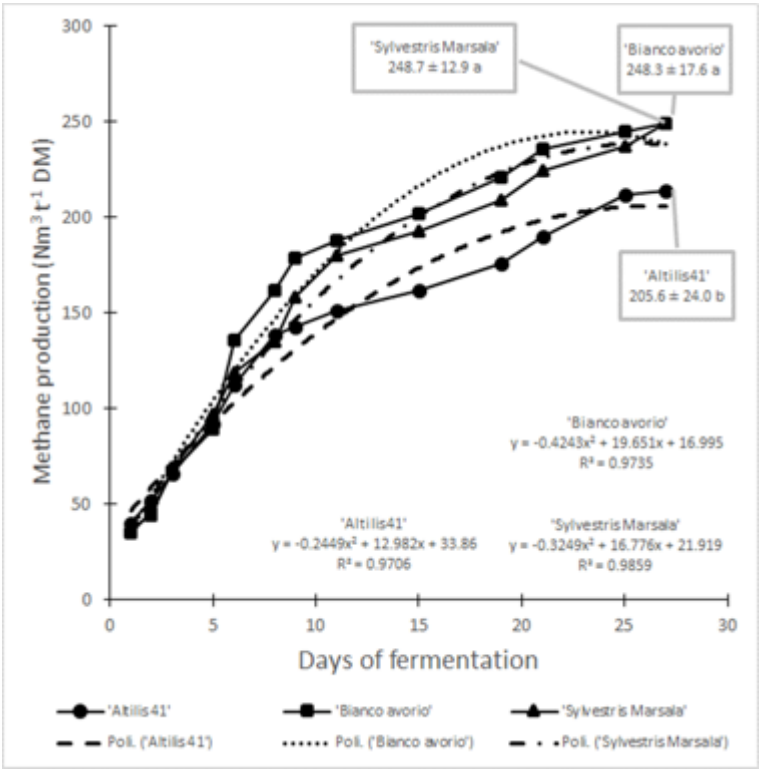

Figure 8.6. Cumulative curves of biomethane production along the fermentation period of three cardoon genotypes in S_3. Data reported in the rectangles are means (N=6) after 27 days of fermentation ± standard deviation. Different letters indicate significance at *LSD* test ($P \leq 0.05$).

In the calculation of biogas production per hectare, ensiling losses were taken into account. However, such losses were not measured, because of the small scale of ensiling in the present trial, but assumed on the basis of experimental experiences on different fodders on a larger scale (Muck and Holmes, 2000, Muck *et al.*, 2003; Muck and Holmes, 2006; Bacenetti and Fusi, 2015). The biomass losses due to ensiling was evaluated in 12% DM. When calculated on a per hectare basis (**Figure 8.7**), the biomethane yield of A41 (4,501 Nm^3 ha^{-1} during S_2, 3,647 during S_3) was not significantly different from those of BA in either S_2 (4,560) or S_3 (3,765) thanks to its higher biomass production per unit area. SM was by far the least productive genotype. Maize silage, which has high starch content, can achieve a biomethane production between 275 and 476 Nm^3 t^{-1} DM (Amon *et al*. 2007, Bauer *et al*. 2010, Negri *et al*. 2014a). The productivity of the cardoon samples was therefore comparable to the lowest biomethane yields achievable by ensiled maize, but was very similar to the levels reported for ryegrass, triticale and wheat. Their silages can generate between 241 and 262 Nm^3 t^{-1} DM biomethane (Murphy *et al*., 2011; Negri *et al*., 2014a; Vítěz *et al*., 2015), and – like cardoon – the DM has a low (or zero) starch content, and is dominated by cellulose. Notably, the biomethane yields achieved from cardoon are in line with those produced from globe artichoke by-products, as detailed by De Menna *et al*. (2016). On a per hectare basis, maize silage generates from 5,300 to 9,000 Nm^3 biomethane (depending on the crop's maturity time and the growing environment) (Kalač, 2011; Bacenetti *et al*., 2014), while the quantity of biomethane derivable from temperate cereals (wheat or triticale) varies from 2,500 to 3,500 Nm^3 and that from ryegrass between 2,000 and 3,000 Nm^3 (Prochnow *et al*.

2009). Comparing BA and A41 (SM was not considered as its biomethane productivity was measured just in S_3), the analysis of variance indicated a strong genotype effect ($P<0.001$) in terms of the biomethane yield per ton of DM. Thus, while BA produced 246 Nm^3 t^{-1} DM per year, on average, the equivalent yield for A41 was only 205 Nm^3 t^{-1} DM. Season significantly ($P<0.001$) affected biomethane yield per hectare, which was 4,530 Nm^3 in S_2 and 3,706 Nm^3 in S_3, on average.

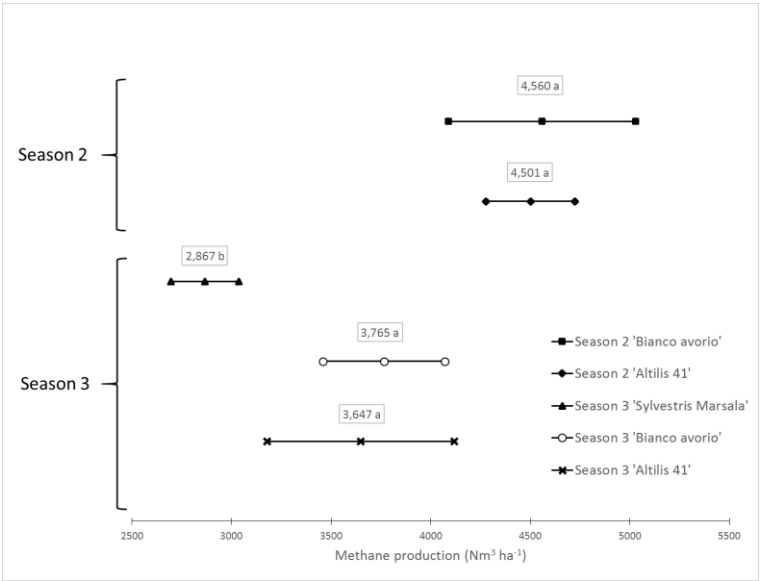

Figure 8.7. Biomethane production per hectare with 95% confidence interval for each genotype in the two seasons. Different letters within each season indicate significance at *LSD* test ($P\leq0.05$).

8.4. Conclusions

The present set of data has confirmed cardoon (and particularly its cultivated form) as a genuine candidate as a renewable source of energy, both when considered in terms of biomass yield and in terms of release of biomethane through anaerobic digestion. The volume of biomethane produced per metric ton of DM was comparable to those produced by ensiled ryegrass, triticale or wheat. When considered on a per hectare basis, cardoon becomes even more attractive given the large level of biomass produced. The levels achieved by the cultivated types averaged 4,530 and 3,706 Nm^3 ha^{-1} in S_2 and S_3, respectively, making them competitive with maize grown under an intermediate input intensity regime. It must be underlined that the cardoons were grown under a zero/minimal input regime with respect to fertilization, irrigation, weed and pest control. To fully exploit the potential of cardoons as a bioenergy crop, focus will be needed to determine their optimal harvesting time in terms of DM and the sugar content of the material. This latter trait in particular has a large influence on biomethane yield through its positive effect on the biomass digestibility.

9. Dilute acid hydrolysis and enzymatic saccharification of field residues of two globe artichoke cultivars.

9.1 Introduction

Globe artichoke is a high biomass producing plant, but the most part of its biomass is a residue. Therefore, the about 15,000 ha planted with globe artichoke in Sicily produce every year a large amount of crop residues represented by plants at the end of productive cycle, consisting in stalks, leaves and unharvested heads. These residues could be an useful feedstock for bioethanol production, even because globe artichoke cultivation is concentrated in 5-6 typical areas, which means the possibility to have large amounts of biomass within a few kilometers. The objective of this trial is to evaluate the suitability of globe artichoke field residues as source of bioethanol without any pretreatment.

9.2. Materials and methods

9.2.1 Climate and soil

The trial was conducted in the area of Cassibile in Sicily (37°03'N, 15°18'E, 10 m asl), where the climate is typically semiarid Mediterranean, with mild winters and hot dry summers. The monthly average maximum temperatures range from 15.7 °C (January) to 32.5 °C (July), while monthly average minimum temperatures from 8.1 °C (January) to 22.2 °C (February). The mean annual rainfall is 515 mm (Servizio Idrografico, 1968-1998). The Peguy climograph (Peguy, 1970) shows (**Figure 9.1**) that the trial location is characterized by temperate months from

November to March, while the arid months are from April to August and, lastly, September and October are warm and humid months. Polygonal area is well developed along the y-axis (rainfall), which means that the local climate is characterized by obvious differences of monthly total rainfall between the autumn-winter months and the spring-summer ones. Such polygon is also elongated in the x-axis (average temperatures), meaning that the local climate is characterized by high annual temperature variations. Meteorological conditions during the trial are described in the Peguy diagram in **Figure 9.2**, from which it can be observed that December, January and February, unlike the long-term period averages (1968-1998), were arid months, notably January was on the border between temperate and arid months (SIAS, 2016). This figure outlines an unusual seasonal trend, which justified the use of irrigation as early as February. The soil is classified as a Calcixerollic Xerochrepts (Soil Survey Staff, 1999), with the following texture: 18.5% clay, silt 26.1%, sand 55.4%.

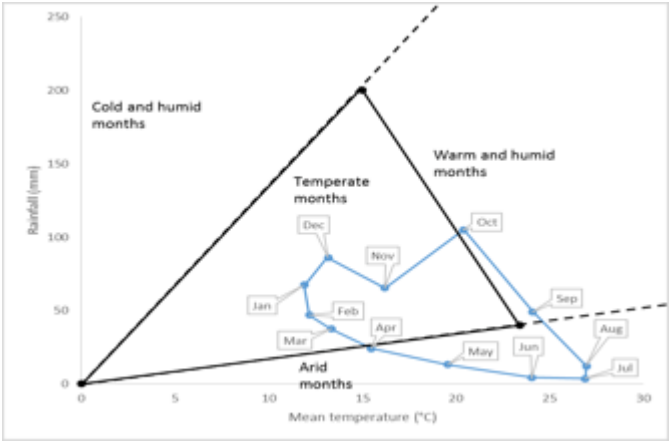

Figure 9.1. Peguy climograph of 1968-1998 mean monthly temperature and monthly precipitation.

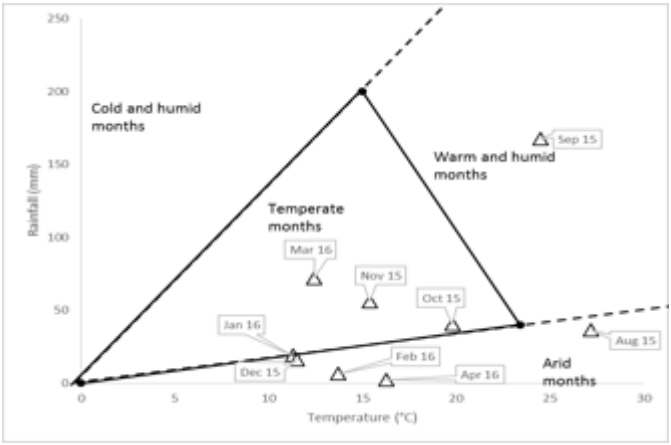

Figure 9.2. Peguy climograph of meteorogical conditions in the trial period (August 2015 - April 2016).

9.2.2 Plant material, crop management and plant sampling

Two globe artichoke genotypes were compared: 'Opera F1', an early purple F1 hybrid specifically bred for annual production, and 'Spinoso sardo', a traditional landrace belonging to the thorny typology. From this point onward 'Opera F1' and 'Spinoso sardo' are referred to as 'Opera' and 'Spinoso', respectively. Crop management was substantially the same for the two genotypes, the only difference concerned the mode of propagation, which was by "seed" for 'Opera' and vegetative (by "ovoli" i.e. underground dried shoots) for 'Spinoso'. 'Opera' "seeds" were sown in the last decade of September, while 'Spinoso' "ovoli" were transplanted at the beginning of August. Prior to planting, the field was ploughed to a depth of ~30 cm and for both genotypes a density of 1 plant m^{-2} was adopted a rate of 1 plant per m^2, using an inter- and intra-row spacing of 1.25 and 0.8 m, respectively. A

fertilization program, commonly used in the area for globe artichoke crops, was adopted [150 and 80 kg ha^{-1} of N and P_2O_5, respectively]. Drip irrigation was carried out when accumulated daily evaporation reached 35 mm, 100% of maximum evapotranspiration (ETP). Pest and weeds control followed standard commercial practice. All inputs were provided according to the crop requirements. In the central part of the field, two blocks were identified, one per each cv. Each block consisted of 3 plots 100 plants. Globe artichoke field residues were collected on April 9th, at that time the harvest was already finished. Field residues, namely plants remaining after harvest and consisting of stalks, leaves and some unharvested heads, were collected in the central area (10 m^2) of each plot by cutting plants at a height of ~5 cm above ground level.

9.2.3 Raw material

Samples of both 'Opera' and 'Spinoso' residues were oven dried at 45 °C to less than 10% of moisture, according to NREL/TP-510-42620 (Hames *et al*., 2008). Then they were milled using a laboratory hammer mill (Retsch GmbH & Co. KG, Germany) and sieved to obtain particles ≥10 mesh for the biomass hydrolysis and fermentation (**Table 9.1**).

Table 9.1. Composition in dimensional classes of 'Opera' raw material.

Dimensional classes	%
From 10 to 18 mesh	55
From 18 to 35 mesh	28
From 35to 60 mesh	10
Less than 60 mesh	7

9.2.4 Chemical characterization of biomass

A portion of these samples were further reduced to 40–60 mesh for the chemical characterization. Samples of both cvs. were characterized and 'Opera' was characterized by analyzing not only the whole biomass, but also each part of plant (leaves, stalks and heads). A Soxhlet's water extraction followed by a Soxhlet's ethanol extraction to prepare extractive-free samples was performed for determination of structural carbohydrates and lignin, according to NREL/TP-510-42618 (Sluiter *et al*., 2012. Extractives and extractive free were analyzed with high performance liquid chromatography (HPLC). The ash content was determined following the standard NREL/TP-510-42622 (Sluiter *et al*., 2008), by calcination of biomass at 525 °C.

9.2.5 High performance liquid chromatography

Sugars, organic acids and ethanol in hydrolysates were analysed by a Merck Hitachi HPLC system (Tokyo, Japan) equipped with refraction index (L7490) and UV-VIS (L7420) detectors. An Aminex HPX-87H (300 x 7.8 mm) cation exchange column from Bio-Rad (USA) was used at 50 °C with H_2SO_4 5 mM with a flow rate of 0.4 mL min^{-1} for quantitative characterization and 0.6 mL min^{-1} for saccharification and fermentation analysis. A Rezex RPM-Monosaccharide (300 x 7.8 mm) was also used at 75 °C with neutral HPLC grade water with a flow rate of 0.6 mL min^{-1} for quantitative characterisation and for saccharification and fermentation analysis. A standard calibration curve was made for each analysed compound.

9.2.6 Dilute acid hydrolysis

Dilute acid hydrolysis was carried out in autoclave at 121 °C, by putting samples in different H_2SO_4, solutions with different liquid/solid ratios.

9.2.7 Colorimetric assays

3,5-dinitrosalicylic acid (DNS) assays were carried out for cellulase characterization and to detect reducing sugars. p-nitrophenyl-β-D-glucopyranoside (PNPG) were carried out for cellobiase characterization. All the absorbance values at the different wavelengths were measured with Helios Alpha UV-VIS spectrophotometer (Thermo Fisher Scientific, Waltham, USA)

9.2.8 Saccharification, enzymes and their characterization

Saccharification was carried out using two enzymes: cellulase from *Trichoderma reesei* and cellobiase from *Aspergillus niger* (Sigma-Aldrich, Saint Louis, USA). Cellulase was characterizated before the saccharification with DNS assays according to NREL/TP-510-42628 protocol (Adney and Baker, 2008). Cellobiase activity was determined through modified Berghem's procedure (Kovacs *et al*., 2009). Saccharification was carried out in 72 hours at 50 °C and at 150 rpm, according to NREL/TP-510-42629 protocol (Selig *et al*., 2008).

9.2.9 Buffering capacity of biomass

Buffering capacity of biomass was measured for 'Opera' at different H_2SO_4 concentrations (0.5, 2.0 and 3.5%) and at different times: before mixing and 30, 60 and 120 minutes from putting together biomass and solutions. pH was

measured with C861 pH meter (Consort, Turnhout, Belgium).

9.2.10 Statistical analysis

For the data subjected to analysis of variance (Snedecor and Cochran, 1989), the means for each trait were separated by Fisher's least significance difference test, applying a threshold of 0.05. Values recorded as percentages were subjected to angular transformation prior to the analysis of variance.

9.3. Result and discussion

9.3.1 Aboveground biomass and its partitioning

As expected, since 'Opera' is a F1 hybrid, it yielded a total aboveground biomass higher (about 17 t DM ha^{-1}) than that of 'Spinoso' (little less than 14 t DM ha^{-1}), although this difference is not statistically significant (**Table 9.2**). 'Opera' showed a weight incidence of residual heads clearly higher than that of 'Spinoso'. 'Opera' showed also a significantly higher incidence of stalks, while 'Spinoso' biomass was characterized by a higher proportion of leaves (**Table 9.2**).

Table 9.2. Above-ground biomass yield and incidence of each part on the total biomass. Different letters within each row indicate significance at *LSD* test ($P \leq 0.05$).

Variable	'Opera'	'Spinoso'
Aboveground biomass yield (t DM ha^{-1})	16.97 a	13.89 a
Heads weight incidence[a] (%)	7.5 a	2.5 b
Leaves weight incidence[a] (%)	35.0 b	57.6 a
Stalks weight incidence[a] (%)	57.4 a	39.9 b

[a] On total of above-ground dry biomass

9.3.2 Characterization of aboveground biomass

Biomass composition is affected by environmental conditions, seasonal trends and cultivation practices, for this reason relating process performance to experimental material composition is very important (Shatalov and Pereira, 2011). 'Opera' and 'Spinoso' have roughly the same composition, but the first one has significantly more ashes. 'Spinoso' was found to be richer in free sugars, glucose and especially fructose (**Table 9.3**).

Globe artichoke residues are characterized by less lignin content and by more extractives than wood species, this means a higher degree of accessibility and therefore reactivity of carbohydrate complex to chemical processing, e.g., in acid hydrolysis, under relatively mild reaction conditions (Shatalov and Pereira, 2011). The contents of cellulose (~27%) and hemicelluloses (~19.8%) in globe artichoke residues found in this study are not so different from those of white corn stover (30% and 19.6%, respectively) (Vargas-Tah *et al.*, 2015). However, values of cellulose and hemicellulose found by Cotana *et al.* (2015) and Fernandes *et al*. (2015) in cultivated cardoon (despite belonging to the same species) are quite different (35-42% and 13-16%) from those found in this study. However, in these latter studies, unlike in the present work, the samples were collected when were already dried (after the flowering and after the summer). Biomass of both cvs. is also characterized by a large amount of extractives and free sugars. This is not without consequences for the purpose of pretreatment, due to the formation of inhibitors. In **Table 9.3** chemical composition of different plant parts of 'Opera' are also shown. The latter has a good potential in producing biomass, hence the

opportunity to better study it by characterizing each part (leaves, heads and stalks). As expected, there is often a statistically significant difference between the different plant parts within the same genotype. Ballesteros *et al.* (2007) studied the suitability of cultivated cardoon stalks for bioethanol production and found that cellulose account for 33.8% of total dry biomass, while hemicellulose for 18.5%. These values are very similar to those of 'Opera' stalks (**Table 9.3**).

9.3.3 Preliminary dilute acid hydrolysis experiments

'Opera' biomass has been subjected to a series of hydrolysis experiments in autoclave at 121 °C for 1 hour, using just water in order to see the its behavior at different liquid/solid (L/S) ratios (10, 12, 15 and 20). After finding that the best (L/S) is 15, other hydrolysis experiments were carried out using two different sulfuric acid concentrations (0.5% and 2% H_2SO_4 solutions). The yield of monosaccharides and decomposition side-products (acetic acid, furfural and hydroxymethylfurfural) was determined in hydrolysates (**Table 9.4)**. As can be seen, pretreatments without sulfuric acid do not cause the degradation of fructose, nor the formation of significant amounts of furans. Arabinose appears in sulfuric acid pretreatments, as product of hemicellulose depolimerization, but without great differences between 0.5% and 2% solutions. The 0.5% sulfuric acid solution coupled with L/S=15, yielded a greater amount of glucose and especially fructose, compared with the water pretreatment with the same L/S, while HMF were present in little more than traces.

Table 9.3. Chemical composition expressed as % of total dry biomass ± standard deviation. Different capital letters within each variable indicate significance at LSD test (P≤0.05) in "Opera all" and "Spinoso all". Different small letters within each variable indicate significance at LSD test (P≤0.05) in the different parts of 'Opera'.

Variable	Opera all		Spinoso all		Opera leaves		Opera stalks		Opera heads	
Ashes	13.93±0.06	A	12.32±0.07	B	29.18±0.66	a	9.42±0.03	b	5.46±0.04	c
EE	2.11±0.05	A	2.11±0.18	A	3.92±0.24	a	1.04±0.13	b	3.36±0.43	a
WE	29.82±0.59	A	31.79±0.26	A	33.06±0.62	ab	27.46±0.54	b	39.44±3.00	a
Glucose	3.21±0.3	B	5.11±0.11	A	Traces		5.44±0.5	a	2.23±0.20	b
Fructose	5.46±0.3	B	8.91±0.21	A	0.94±0.01	b	9.81±1.8	a	6.21±0.50	a
Glucan	26.24±0.09	A	28.37±0.84	A	19.37±0.01	b	31.66±1.54	a	24.82±1.58	b
Hemicellulose	19.74±0.61	A	19.81±0.01	A	14.17±0.04	c	23.80±0.46	a	19.99±0.36	b
Xylan	13.11±0.35	A	13.12±0.03	A	8.33±0.01	c	15.83±0.43	a	13.41±0.35	b
Arabinan	5.59±0.19	A	5.51±0.01	A	5.70±0.05	b	6.07±0.07	a	5.34±0.03	c
Acetic acid	1.27±0.08	A	1.18±0.02	A	0.64±0.03	c	1.91±0.03	a	1.24±0.04	b
KL*	10.73±0.01	B	10.99±0.03	A	13.75±0.13	a	10.99±0.03	b	8.27±0.11	c
Others	11.36		6.93		15.73		5.04		4.13	

"Opera all" and "Spinoso all" refer to the whole 'Opera' and 'Spinoso' biomasses, respectively. "Opera leaves", "Opera stalks" and "Opera heads" refer to the different part of plant of 'Opera'.
WE=water extract; EE=ethanol extract; KL= Klason lignin
*contains also the proteins

Table 9.4. Monosaccharides and decomposition side-products of liquid fraction coming from preliminary hydrolysis experiments on 'Opera' biomass. Results are expressed as % of dry matter. Different letters within each variable indicate significance at LSD test (P≤0.05).

Liquid phase	L/S	Glu	Fru	Xyl	Ara	AcA	HMF	F	RS
H_2O	10	3.8b	6.0b	-	-	0.2c	traces	traces	71.3b
H_2O	12	3.3cd	5.4c	-	-	0.2c	traces	traces	77.1a
H_2O	15	3.5cd	5.8bc	-	-	0.2c	traces	traces	71.2b
H_2O	20	3.1d	5.5c	-	-	0.2c	traces	traces	68.2b
H_2SO_4 0.5%	15	3.8b	9.2a	-	1.2a	0.6b	0.3b	traces	61.9c
H_2SO_4 2%	15	4.3a	-	10.9	1.4a	2.4a	1.8a	0.1	53.0d

Glu=glucose; Fru=fructose; Xyl=xylose; Ara=arabinose; AcA=Acetic acid; HMF=hydroxymethylfurfural; F=furfural; RS=recovered solids

With 2% sulfuric acid solution and L/S=15, glucose increased (due to partial hydrolysis of cellulose), fructose disappeared and a large amount of xylose, coming from hydrolyzation of hemicelluloses, appeared in the composition of liquid fraction. On the other hand, the highest sulfuric acid concentration caused a significant decomposition of hexoses into hydroxymethylfurfural and certainly a higher acetic acid production. Furfural was found in smaller quantities, as sign of a modest degradation of xylose. Generally, increase in reaction temperature and acid concentration, while accelerating xylan hydrolysis to xylose, intensifies substantially the secondary degradation reactions of monomeric sugars (Shatalov and Pereira, 2011). The content of free sugars decreased as the L/S increased, while the amount of solid recovered seemed not so much to be related to the L/S, as to the acid concentration.

9.3.4 Water pretreatment and dilute acid hydolysys

Since the biomass is rich in extractives and free sugars (hexoses) (**Table 9.3**) which demonstrated to be easily degraded as hydroxymethylfurfural (**Table 9.4**), it seemed appropriate to implement a water pretreatment to wash away the free sugars that can be directly transformed into inhibitors during the dilute acid hydrolysis. Samples of 'Opera' were put in water at two different L/S (10 and 15) and heated in autoclave at 100 °C for 15, 30, 45 and 60 minutes, in order to identify the L/S-duration combination which yields the highest amount of free sugars. As shown in **Table 9.5**, the best combination was 15 minutes and L/S=10, which is preferable also because employs less energy and water. A dilute acid hydrolysys experiment was carried out to verify the water-pretreatment effect on total soluble sugars and inhibitors in the liquid fraction (**Figure 9.3**) and it proved to be very effective in reducing hydroxymethylfurfural (0.2% against 1.8% of non-water-pretreated material), while it did not

change the furfural content and increased the acetic acid. Regarding sugar yield, as expected, glucose content was lower (0.5% against 4.3%), because the monomeric glucose was previously washed away, therefore the glucose obtained after the dilute acid hydrolysis came from the hydrolyzation of cellulose. Xylose content was lower (8.6% against 10.9%), but it could be justified by the natural variability of the biomass.

9.3.5 Buffering capacity of 'Opera'

Since the basic cations of lignocellulosic biomass can partially neutralize the sulfuric acid (Esteghlalian *et al.*, 1997) and since globe artichoke residues are rich in ashes (**Table 9.3**), the acidity of reaction mixture is lowered by the buffer capacity of biomass. An experiment to test the buffer capacity of 'Opera' was carried out by mixing different sulfuric acid solutions (0.5, 2 and 3.5%) with the biomass. pH was measured at room temperature (~25 °C) before mixing and after 30 min. (T1), 1 hour (T2) and 2 hours (T3).

Table 9.5. Free sugars content of the liquid coming from the water pretreatment of 'Opera' expressed as % of total dry biomass.

Time (min)	L/S	Glu	Fru
15	10	4.46	4.78
15	15	3.65	4.64
30	10	4.04	4.65
30	15	3.67	4.69
45	10	3.87	4.47
45	15	3.59	4.54
60	10	3.91	4.42
60	15	3.62	4.54

L/S= liquid/solid ratio; Glu=glucose; Fru=fructose

The L/S was 15 (**Figure 9.4**). Results show that at 3.5% [H^+] do not decreases at T1 (on the contrary slightly increases), but then at T2 it lowers by 20% and finally after two hours (T3) increases, but without reaching the value before mixing. A similar behaviour is showed by the reaction mixture with 2% H_2SO_4, even if [H^+] gradually decreases from the before mixing value to the value observed after two hours from mixing. The buffering capacity of the biomass is more effective at low acid concentration, indeed at 0.5% [H^+] it already decreased by 50% after half an hour and then it remained stable.

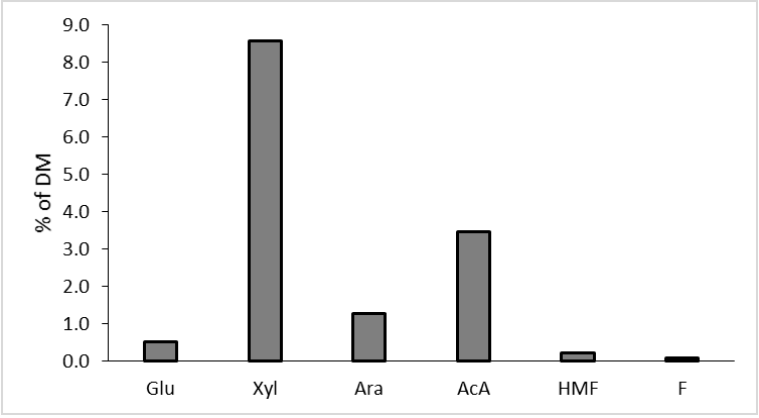

Figure 9.3. Monosaccharides and decomposition side-products of liquid fraction coming from dilute acid hydrolysis of water-pretreated 'Opera' biomass. Results are expressed as % of dry matter. Glu=glucose; Xyl=xylose; Ara=arabinose; AcA=Acetic acid; HMF=hydroxymethylfurfural; F=furfural

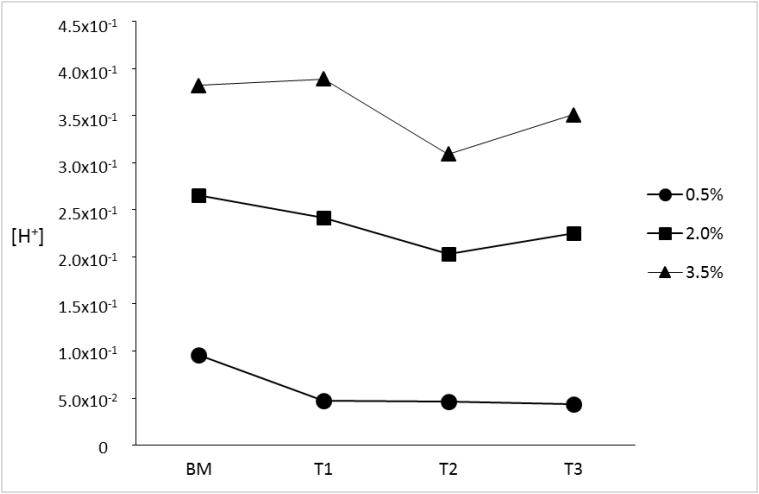

Figure 9.4. Buffering capacity of 'Opera' biomass measured as $[H^+]$ at different H_2SO_4 concentrations (0.5, 0.2 and 3.5%) at different times (BM=before mixing; T1=half an hour; T2=1 hour; T3=2 hours). L/S=15.

9.3.6 Saccharification of non pretreated 'Opera' biomass and its potential in bioethanol production

Glucose yields of liquid fraction coming from untreated 'Opera' and 'Spinoso' after 72 h of enzymatic saccharification are reported in **Table 9.6**. Both cvs. achieved very high values (~27% and ~23%, respectively), higher than those obtained from cultivated cardoon by Fernandes *et al*. (2015) (~11%), Ballesteros *et al*. (2008) (~14%), Shatalov and Pereira (2011) (~19%) and Martinez *et al*. (1990) (~20%). Leaves were the part of 'Opera' which by far yielded more glucose. **Table 9.6** also reports the yields in bioethanol of 'Spinoso' and 'Opera', obtainable after saccharification. The yield in bioethanol was estimated taking into account not only the glucose coming from the cellulose saccharification, but also the free sugars (glucose and fructose). 'Spinoso' was significantly more productive than 'Opera' both on dry matter basis (~109 kg of bioethanol per t

DM vs. ~85) and on a per hectare basis (~1519 kg ha^{-1} vs. ~1438 kg ha^{-1}), in spite of the higher biomass productivity of 'Opera'. The reason for this difference lies on the greater content of free sugars in the 'Spinoso' than in the 'Opera' biomass (**Table 3**). In **Table 6**, data of 'Opera' are also separated by plant part and it can be seen that leaves were the most glucose yielding parts (from saccharification), but stalks' contribution in bioethanol production was higher, because of their content in free sugars. (**Table 3**).

Table 6. Yield of glucose (% of glucan) and bioethanol (kg t-1 DM and kg ha-1) obtainable after enzymatic saccharification of non-pretreated biomass. Different capital letters within each variable indicate significance at LSD test (P≤0.05) in "Opera all" and "Spinoso all". Different small letters within each variable indicate significance at LSD test (P≤0.05) in the different parts of 'Opera'.

Variable	Glucose yield (%)	Bioethanol (kg t^{-1} DM)	Bioethanol (kg ha^{-1})
Opera all	27.1±0.57 A	84.7±0.86 B	1438.3±14.62 B
Spinoso all	23.3±0.19 B	109.3±0.30 A	1518.8±4.22 A
Opera leaves	50.9±0.16 a	60.8±0.17 c	361.1±1.04 B
Opera stalks	20.7±0.82 b	115.2±1.48 a	1122.4±14.43 A
Opera heads	24.6±1.82 b	77.8±2.57 b	99.1±3.26 C

"Opera all" and "Spinoso all" refer to the whole biomass of 'Opera' and 'Spinoso ' biomasses, respectively. "Opera leaves", "Opera stalks" and "Opera heads" refer to the different part of plant of 'Opera'.

9.4 Conclusions

The field residues of globe artichoke represent a ligno-cellulosic biomass which is abundant (17.0 t DM ha^{-1} for 'Opera' and 13.9 t DM ha^{-1} for 'Spinoso'), rich in complex carbohydrates (~27% of cellulose and ~19.8% of hemicelluloses) and relatively rich in simple sugars: glucose (3.21% for 'Opera' and 5.11% for

‘Spinoso’) and especially fructose (5.46 for ‘Opera’ and 8.91 for ‘Spinoso’). The richness in simple sugars is an advantage, but requires the use of expedients to avoid them to be directly transformed into inhibitors. For this reason a water extraction was implemented, to wash away simple sugars before dilute acid hydrolysis. From the high content of ashes comes the buffering capacity of globe artichoke biomass, for this reason the experiment of dilute acid hydrolysys with low concentration H_2SO_4 (0.5%) was not effective in hemicellulose depolimerization. The enzymatic saccharification of non-pretreated biomasses of the two globe artichoke cvs. has given rise to estimated yields of ~85 kg of bioethanol per t DM for ‘Opera’ and ~109 kg of bioethanol per t DM for ‘Spinoso’. On a per hectare basis, estimated bioethanol yield for ‘Opera’ is ~1438 kg ha^{-1}, while the one for ‘Spinoso’ is ~1518 kg ha^{-1}.

10. Concluding remarks

Globe artichoke, which plays an important role among the field-scale crops cultivated in the Mediterranean Basin, as minimally processed and ready-to-use product can enhance its consumption. Results demonstrate that both biochemical and physical parameters of ready-to-use globe artichoke slices may be affected by several factors including genotype, harvest time, storage time and packaging. Overall, ready-to-use globe artichoke slices maintained high nutritional quality and colour parameters for at least 7 days of storage. Nevertheless, the fluctuation observed for both harvest time and storage time was found to be linked to the genotype. Based on such consideration, 'Exploter' was found to be the most suitable for processing as ready-to-use globe artichoke slices, especially in the later harvests. Regarding the packaging, results showed that Cast Polypropylene film achieved better performances than compostable BIObased film. However, the latter is interesting in order to reduce wastes and the environmental impact.

Cultivated and wild cardoon optimize the native resources of Mediterranean environment and, at the same time, endure its harshness. Their low input requirements and their capacity to be grown in non-arable land result in a low environmental pressure and in the ethical opportunity of avoiding competition with food crops. The data presented here confirmed that cardoon, especially the cultivated one, could be a very competitive renewable energy crop in terms of aboveground biomass yield and for transformation in biomethane through anaerobic digestion. Biomethane production per ton of DM of cardoon silage proved to reach a satisfactory level, which is comparable to those of silage of autumn-spring fodder crops and cereals, such as ryegrass, triticale and wheat. The potentiality in terms of biomethane production per hectare is interesting too. Cultivated

cardoon silage yielded about 5,150 (S_2) and 4,200 (in S_3) Nm^3 ha^{-1}, these values are similar to those of early varieties of corn grown under middle-high inputs. This potential has even greater interest, if we take into account that cardoon was grown under zero/minimal inputs as fertilization, irrigation, weed and pest control. This demonstrates the high ability of cardoon to reduce environmental impact developing a new sustainable model of bioenergy crops.

The great amount of crop residues left in field by globe artichoke crops could be exploited for bioethanol production. Globe artichoke residues are well suited for this purpose not only for their ligno-cellulosic biomass, but also for their richness in simple sugars. Enzymatic saccharification of non-pretreated biomass proved to yield a large amount of fermentable sugars, but further studies are needed in order to find the best pretreatment for optimizing the sugar yield, with the lowest possible content of inhibitors.

As a whole, the results of this study encourage alternative uses of *C. cardunculus* L. especially in Mediterranean environment.

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