UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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EVALUATION OF FRUIT QUALITY IN A COLLECTION OF PEPPER LANDRACES (Capsicum annum) UNDER ORGANIC AGROFORESTRY CONDITIONS IN PORTUGAL

Master's Thesis

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EVALUATION OF FRUIT QUALITY IN A COLLECTION OF PEPPER LANDRACES (*Capsicum annum* L.) UNDER ORGANIC AGROFORESTRY CULTIVATION

ERASMUS MUNDUS MASTER'S IN PLANT GENETIC IMPROVEMENT

(emPLANT)

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

MASTER THESIS

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ABSTRACT

Nowadays, the global demand for nutritionally healthier and environmentally friendly food production has led to a rapid transformation in organic agriculture. Local varieties with broad genetic diversity have become resources for organic cultivation due to the increased consumption of organic products. In Spain, peppers are highly valued for their economic significance and bioactive compounds that offer health benefits for consumers. Understanding the impact of organic agroforestry farming on pepper fruit quality, particularly their content of bioactive compounds, is crucial for the organic market. This study evaluated five Spanish landraces of *Capsicum annuum* under organic agroforestry conditions in Portugal in the frame of the project LIVESEEDING. In addition to agronomic traits, the levels of main flavonoids, total phenolics, sugars, carotenoids, and ascorbic acid contents of pepper fruits were analyzed using HPLC and spectrophotometry, at the UPV-COMAV laboratory. Two ripening stages were considered, green ripe and fully ripe dry fruits. Carotenoids were assessed only at the fully ripe stage. The statistical analysis was performed using Stat Graphics 18 software. The analysis of variance (ANOVA) was used and the Student-Newman-Keuls test was utilized for mean separation at a significance level of $p \leq 0.05$. Significant effects were observed for variety, ripening stage, and their interaction regarding fruit diameter, fruit weight, number of locules, and total yield. The analysis of bioactive compounds revealed that quercetin (61%) and luteolin (32%) were the primary contributors to total flavonoids at both ripening stages. The ripening process generally increased the levels of individual flavonoids, except for quercetin and overall, for total phenolics. Glucose and fructose were the major contributors to total sugar, particularly at the fully ripe stage. Noteworthy interaction effects were observed for ascorbic acid, and there was a significant varietal effect on red and yellow carotenoids, which ranged between 1019-3121 µg/g DW. Positive correlations were found between flavonoids, especially between quercetin and kaempferol as well as between luteolin and apigenin ($p > 0.8$). These findings emphasize the significance of organic farming, particularly under agroforestry conditions, for producing peppers with high-value-added bioactive compounds, in addition to the influence of variety and ripening stage. This approach aligns with the growing concern for food quality among the global population.

Keywords: *Capsicum annuum*; organic agroforestry; landraces; phenols; bioactive compounds.

RESUMEN

En la actualidad, la demanda mundial de una producción de alimentos más sanos desde el punto de vista nutricional y respetuosos con el medio ambiente ha provocado una rápida transformación de la agricultura ecológica. Las variedades locales con amplia diversidad genética se han convertido en recursos para el cultivo ecológico debido al aumento del consumo de productos ecológicos. En España, los pimientos son muy valorados por su importancia económica y sus compuestos bioactivos que ofrecen beneficios para la salud de los consumidores. Comprender el impacto del cultivo agroforestal ecológico en la calidad de los frutos de pimiento, en particular su contenido en compuestos bioactivos, es crucial para el mercado ecológico. Este estudio evaluó cinco variedades autóctonas españolas de Capsicum annuum en condiciones agroforestales ecológicas en Portugal en el marco del proyecto LIVESEEDING. Además de los rasgos agronómicos, se analizaron los niveles de flavonoides principales, fenólicos totales, azúcares, carotenoides y contenido de ácido ascórbico de los frutos de pimiento mediante HPLC y espectrofotometría, en el laboratorio de la UPV-COMAV. Se consideraron dos estados de maduración, frutos verdes maduros y frutos secos completamente maduros. Los carotenoides se evaluaron sólo en el estado de maduración completa. El análisis estadístico se realizó utilizando el programa Stat Graphics 18. Se utilizó el análisis de varianza (ANOVA) y la prueba de Student-Newman-Keuls para la separación de medias a un nivel de significación de $p \leq 0,05$. Se observaron efectos significativos de la variedad, el estado de maduración y su interacción en el diámetro del fruto, el peso del fruto, el número de lóculos y el rendimiento total. El análisis de los compuestos bioactivos reveló que la quercetina (61%) y la luteolina (32%) eran los principales contribuyentes al total de flavonoides en ambas fases de maduración. El proceso de maduración aumentó en general los niveles de flavonoides individuales, excepto para la quercetina y, en general, para los fenoles totales. La glucosa y la fructosa fueron los principales contribuyentes al azúcar total, especialmente en la fase de maduración completa. Se observaron notables efectos de interacción para el ácido ascórbico, y hubo un significativo efecto varietal sobre los carotenoides rojos y amarillos, que oscilaron entre 1019-3121 µg/g DW. Se encontraron correlaciones positivas entre flavonoides, especialmente entre quercetina y kaempferol, así como entre luteolina y apigenina ($p > 0,8$). Estos resultados enfatizan la importancia de la agricultura ecológica, especialmente en condiciones agroforestales, para producir pimientos con compuestos bioactivos de alto valor añadido, además de la

influencia de la variedad y el estado de maduración. Este enfoque se alinea con la creciente preocupación por la calidad de los alimentos entre la población mundial.

Palabras clave: Capsicum annuum; agroforestería orgánica; variedades locales; fenoles; compuestos bioactivos.

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1. INTRODUCTION

Pepper is an annual herbaceous plant under the genus *Capsicum* in the *Solanaceae* family, that contains at least 32 species native to tropical America (Hernández-Pérez et al., 2020). The genus *Capsicum* includes chili peppers, bell peppers, ajíes, habaneros, jalapeños, ulupicas and pimientos, well known for their economic importance around the globe and consumed by one fourth of the global population (Swammy, 2023). Peppers can be consumed raw (e.g. bell peppers), or in powdered form as a spice (chili pepper) or as a food colorant (paprika). The fruits can have different colors from green, yellow, red, orange and corresponding to their distinct stages of maturation and carotenoids and chlorophylls synthesizing capacities (Baenas et al., 2019).

The main specificity of pepper is its pungency characteristics due to secondary metabolite capsaicinoids produced only in *Capsicum* peppers (Jr et al., 2007). In addition to, sensory features such as pungency, aroma, and color pepper are important sources of bioactive compounds that offer health benefits for consumers, including vitamin C and E, provitamin A, phenolic compounds mainly flavonoids and phenolic acid derivatives, anthocyanins, carotenoids and antioxidant activity (Padilha et al., 2015). Surprisingly, phenolic compounds found in capsicum are helpful in preventing and treating many ailments. In the pharmaceutical industry, it intends as a beneficial milestone and a boon to humanity (Swammy, 2023).

In terms of medicinal properties, these bioactive compounds found in *Capsicum* fruits have a role of antioxidant, anticarcinogenic, anti-inflammatory (capsaicinoid), prevention of cancer, atherosclerosis (phenolic compounds), prevention of gastric ulcers (like luteolin), cardiovascular disease and age related macular degeneration cataracts (carotenoids) (Mendes et al., 2020). In addition, ascorbic acid plays important role in preventing scurvy, coronary artery and kidney disease and vitamin E as well protects approximately 80 diseases by scavenging free radicals, preventing anemia, diabetes, cardiovascular diseases and others (Mendes et al., 2020).

Pepper derived ingredients also play as natural ingredients instead of synthetic preservatives that could enhance the health properties of a great role in cosmetics and pharmaceutical products, avoiding the contact allergies caused as side effects, antiaging effect (major product claims from

the cosmetic industry) and wrinkles by fighting against free radicals and solar radiation (Baenas et al., 2019).

1.1 Economic Importance

Pepper production worldwide has grown considerably over 20 years (2001-2021, www.fao.org/faostat), from 2.4 to 4.8 million tons of dry types and from 21.4 to 36.2 million tons as fresh chilies and peppers. The area harvested being today 3.6 million of hectares, followed a similar trend with an increase of surface cultivated area about 28% in the last 20 years. Fresh pepper is cultivated in 127 countries of the world in all the continents. China is the world's largest producer annually production of more than 16 million tons (Figure 1), followed by Turkey and Indonesia with about 3 million tons (FAOSTAT, 2023).

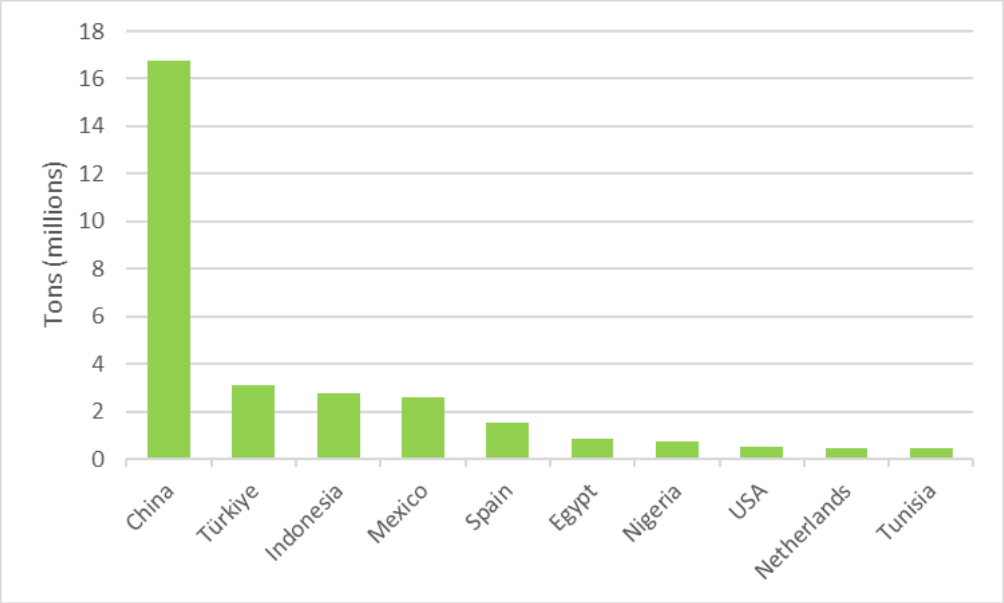


Figure 1. Top ten producers of green chilies and peppers (Source, FAO 2023).

Dry pepper is cultivated in 58 countries, India is the largest producer with production quantity of 2 million tons followed by Bangladesh (0.49 million tons) as shown in Figure 2. Currently, peppers are grown almost all over the world and equitably easy to cultivate both in open field and in greenhouse in a wide range of climatic and environmental conditions.

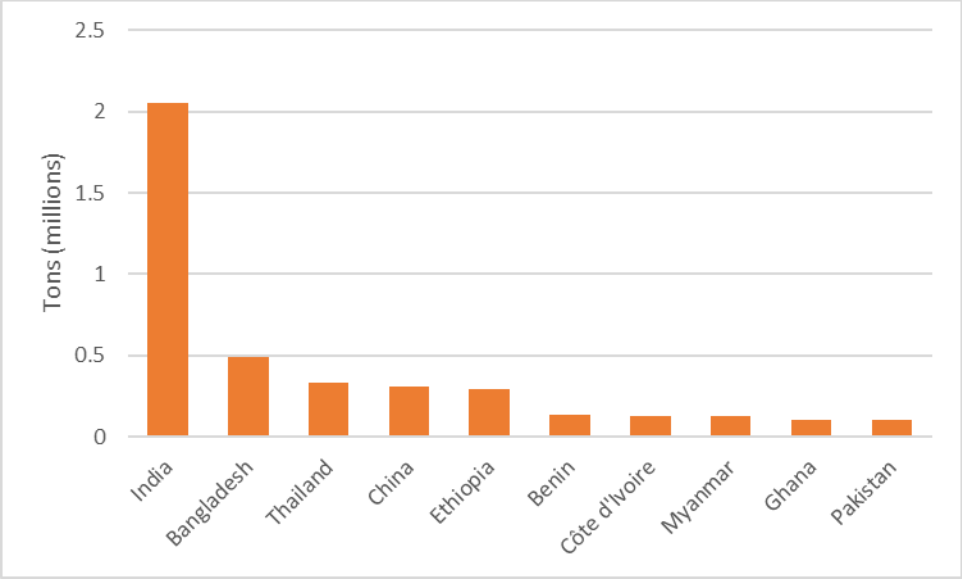


Figure 2.Top ten producers of dry chilies and peppers (Source, FAO 2023).

Approximately, more than 67% of total green chilies and peppers production in the world is located on the Asian continent with the main growing areas located in China and Indonesia (Figure 1). Africa, Europe, and America contribute in the proportion to the total world production about 10-13% for fresh pepper, while for dry pepper Asia and Africa are the main producers contributing to 73% and 21%, respectively (Figure 3).

In 2021, the EU chili and pepper market increased by 2.2% and the level of consumption peaked in 2021 and expected to retain growth in years to come. Spain is the largest producer and exporter of chilies and peppers in the European Union, with the volume exports recording 879K tons which was 55.4% of total exports under European union in 2021. It was distantly followed by Netherlands (450 k tons), generating a 28% share of total exports. Total exports of 9.5% together made up from France (52 k tons), Belgium (40 k tons), Austria (34 k tons) and Hungary (24 k tons). In terms of value, Spain (1550 million $), The Netherlands (1142 million $), and France (88 million $) were the top three exporters in 2021, with a combined share of 88% total exports.

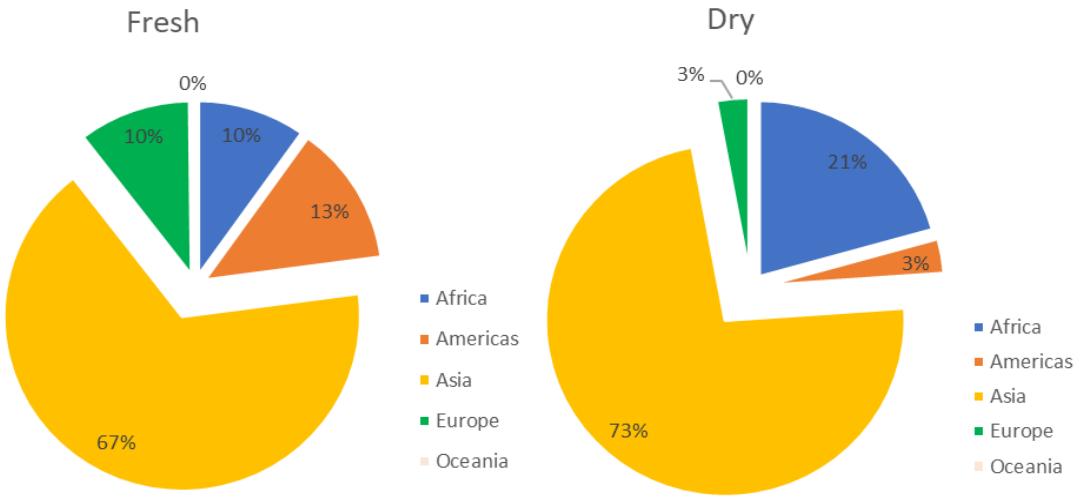


Figure 3. Production share of fresh and dry Chilies and peppers (FAOSTAT 2023).

Germany was the main importer of chilies and peppers, contributing nearly 30% of total imports in 2021. France (12.8%) took the second position followed by the Netherlands (8.6%), Italy (5.8%), Spain (5.3%), Poland (4.8%) and 32.7% share of total imports were accounted from the remaining countries. Regarding import value, Germany was accounting the highest value (1015 million $), fairly followed by France (308.7 million $), The Netherlands (233 million $) and Italy, Spain and Poland together contributed 16% combined share of the total imports (Table 1).

Table 1.Top European countries in green chilies and peppers exports and imports market.

Country	Exports Quantity (Tons)	Export Quantity (%)	Export Value (million $)	Country	Imports Quantity (Tons)	Import Quantity (%)	Import Value (million $)
Spain	879028	55.4	1550.4	Germany	431123	29.8	1015.2
Netherlands	450892	28.4	1142.7	France	185518	12.8	308.7
France	51938	3.3	87.6	Netherlands	124273	8.6	232.8
Belgium	40382	2.5	76.6	Italy	84307	5.8	126.6
Austria	34275	2.2	58.1	Spain	76400	5.3	81.6
Hungary	24048	1.5	37.7	Poland	69407	4.8	138.9
EU	1587739		3155.7	EU	1448868		2781

Similarly, Spain was the first for the export of dry chilies and peppers, with the export volume 81K tons accounting 71.6% under European unions in 2021. The Netherlands is the second country achieving export quantity of 8.6 K tons, achieving 7.6% total share of exports (Table 2). Germany, Hungary, Belgium, Slovenia, and France together contributed 21.8% to the total export quantity of volume of dry chilies and peppers. Spain stands out with more of export value (250 million $), from total European countries export value (423 million $). Then The Netherlands followed by 38 million $ as usual, Germany (48 million $), Hungary (12.5 million $), Belgium (9.5 million $), Slovenia (4.6 million $), France (13.2 million $).

Table 2.Top European countries in dry chilies and peppers, exports and imports market.

Country	Exports Quantity (Tons)	Export Quantity (Tone) %	Export Value (million$)	Country	Imports Quantity (Tons)	Import Quantity (%)	Import Value (million$)
Spain	81413	71.6	250.5	Spain	72329	43.7	146.2
Netherlands	8692	7.6	38.3	Germany	26371	15.9	91.6
Germany	7776	6.8	48.4	Netherlands	15705	9.5	44
Hungary	2443	2.2	12.5	Poland	7445	4.5	29.4
Belgium	2068	1.8	9.6	France	7131	4.3	25.2
Slovenia	2022	1.8	4.7	Austria	4354	2.6	17.7
France	1803	1.6	13.2	Hungary	4169	2.5	12.5
EU	113629		422.6	EU	165412		474.7

In terms of import quantity of dry chilies and peppers, Spain is the leading country by accounting 43.7% import quantity of 72 k tons with 146 million $ import values under European union. Germany was the second higher import quantity (15.9%) generating 91 million $ import value, The Netherlands (9.5%) import value of 44 million $, Poland (4.5%) with 29.4 million $, France (4.3%) value of 25 million $, Austria (2.6%) import value 17.7 million $ and Hungary import quantity of 2.5% with value 12.4 million $ of total European union in 2021 (Table 2).

1.2 Taxonomic and Morphology of Pepper (Vegetative and Reproductive)

Solanaceae is a complex and cosmopolitan family comprised of at least 98 genera and as many as 2716 species including *Capsicum*, an economically important plant (Eshbaugh, 2012). Most of the *Solanaceae* family members have the same chromosome number 2n=2x=24 including the domesticated and the wild species, while genome size varies significantly from one species to another. Chromosome number of 2n=2x=26 reported in a few species (Eshbaugh, 2012) and only one reference to natural wild polyploidy accession of *C. annuum* with 2n=48 chromosome is available (Baran Jha et al., 2012). The *Capsicum* genus encompasses more than 40 species with wide variability in shape, size, color, and sensory attributes (Eshbaugh, 2012). The five major and domesticated species of *Capsicum* include *Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens,* and *Capsicum pubescens.* The *C. annuum* genome size (3.48 GB) is around three to four times larger than tomato (*Solanum lycopersicum*; 0.76 GB) and potato (*S. tuberosum*; 0.73 GB) (Sampaio et al., 2023).

The floral formula of the family characterized by calyx, K (3-5); corolla, C (5-8); androecium A (5-8), and gynoecium, G (2-4) with herbaceous, short bushes with a woody stem and bright fruits of red, green, orange, chocolate, or yellow color (Devi et al., 2021).

Figure 4. *Capsicum annuum var. annuum.* A. flower bud on pendent pedicel; B. flower with connivent anthers; C. flower with hexamerous corolla (note nectar droplets on the limb) and style near the same length as the anthers; D. flower with heptamerous purple coroll; E. flower with pentamerous corolla and style exceeding the anthers; F. mature fruits on pendent pedicels; G. mature fruits on upright pedicels); H. mature fruits on pendent pedicels (Swammy, 2023).

The leaves known by their alternate and elliptical nature with having perfect and complete flowers comprised of a calyx, corolla, and male and female reproductive organs. The flower is characterized by : 5-7 stamens, pale blue to purplish anthers that have been observed to open at 05:00-06:00 h and anther dehiscence occurs at 08.00 to 11:00 h based on variety and climate (Devi et al., 2021).

Self-pollination is the main mode for fruit formation (Kuhn et al., 2016). However some amount of outcrossing due to insect activity (Putra et al., 2016). Anthesis usually occurs after flower opening, and the flower remains open for 2-3 days. Flower opening starts at 7:00 a.m. which continues up to 11:00 a.m., but the peak time is between 7:15 a.m. and 10: 00 a.m. in the case of sweet pepper. The anthers dehiscence after 30 min of flower opening. In the day of anthesis, pollen viability and stigma receptivity are maximum, but the stigma remains receptive up to 2 days after anthesis. Although, flower opening delays during cold and cloudy days (Sharma et al., 2020).

1.3 Origin and Distribution

The genus *Capsicum* is native to tropical and subtropical America, comprising Mexico and Northern Central America, the Caribbean, the lowland Bolivia, the Northern lowland Amazonia and the mid-elevation Southern Andes (Perry et al., 2007). Mexico is the center of diversity for the most important cultivated species *Capsicum annum L*. Southern Texas and Argentina are also the place for their wild forms of this species (Welbaum, 2015).

Capsicum is a genus comprised of more than 40 species with at least 5 taxa from southeastern Brazil and several new species described in Bolivia (Eshbaugh, 2012), many authors ascribing 20 to 50 species to the genus with reported 40 accepted species (Swammy, 2023). Among these five of them are domesticated: *C. annuum, C. frutecenses L., C. chinese Jacq., C. baccatum L., and C. pubescens.* *C. annuum* initially domesticated in Mexico or northern central America (Perry et al., 2007). *Capsicum annum* is the most economically important species throughout the globe among the five domesticated due to its pungent oduor, taste and a wide range of flavors and intensities from sweet to mild to hot (Swammy, 2023). The center of diversity for *C.*

c*hinense* and *C. frutescens* comprises on the Amazonian regions of South America, with secondary centers in Central America and Caribbean islands (Welbaum, 2015). *C. baccatum* including their complex *C. praetermissium* and *C. tovarii*, diversity is greatest in low to mid-elevation regions of Bolivia, and the primary centers of diversity for *C. pubescens* are Peru and Brazil, south of the Amazon basin and mid-elevation, Andean regions of Bolivia and Peru (Welbaum, 2015).

The pepper crop introduced into Spain by Colombus in 1493. In 1548, its cultivation spread from the Mediterranean region to England and to Central Europe by the close of the 16th century and it came in China under cultivation during the late 1700s (Swammy, 2023).

1.4 Cultivation and Management of Pepper

1.4.1 Climate Requirements and Growing Season

Climatic factors have a great impact on seed germination, development, and fruiting of pepper plants. Pepper is a tropical, that requires high temperatures throughout their growth cycle and its sensitive to low temperature and frost condition. It can be grown both as an open field crop and as a protected greenhouse crop. Under open field condition, grown in hills during summer and during winters in plains in the case of bell peppers. In the case of open field condition sweet pepper requires long and warm growing season , which is frost free for 4-5 months and it can be grown throughout the year under protected condition (Sharma et al., 2020).

Temperature range between 21 and 30 ℃, with an average of 18 ℃, is the ideal temperature for best growth of pepper. Low temperature leads to decrease in germination, wilting plant parts and slow growth. On the other hand, high temperature above 35 ℃ have an impact on fruit set, especially with low air humidity or dry winds (do Rêgo et al., 2016). There is an impact of high and low temperatures in fruit quality, especially sugar content and vitamin C, in addition the intensity of red and yellow colors, they are generally greater when the temperature increases. There is also an evidence that indicate low temperature can affect the pungency of pepper, Spring-Summer grown more pungent than Autumn- Winter (do Rêgo et al., 2016).

1.4.2 Soil Preparation, Seedling Production and Fertilization

The most suitable soil for pepper production is medium clay to sandy texture; heavy/compacted and sandy soil should be avoided. It is not advisable to plant in areas where that have been grown the same pepper family as tomato, potato, eggplant, and the like in the previous year as peppers are susceptible to attack by pests and diseases. So, crop rotation with beans or corn is recommended to avoid continuous planting for years on the same site. Good soil preparation is also important to facilitate setting and rooting of plants and seedlings have to be spaced from 1.2 to 1.5 m (do Rêgo et al., 2016).

Seedling production can be trays of 128 cells, filled with commercial substrates containing two or three seeds per cell. Although the most used are polystyrene trays, have advantage of being nonporous, preventing chemical and biological contamination and can be washed with high pressure water with greater efficiency. Plastic bags with volume between 150 and 250 cm^3, filled with three parts soli previously treated with limestone applied by some producers. Nursery bed is also one of the oldest and cheapest method for seedling production, with bead size of 1.0-1.2 m a wide and 0.2-0.25 m high. An average of 2-3 g seeds per square meter of bed can be used and should be covered with a thin layer of soil after the distribution of the seed. Putting dry straws or grass prevents the impact of water drops and rain to reduce germination and seedling emergence. Strong sunlight should be reduced from seedlings by 50% incident radiation. Then, it is necessary to remove the cover after the seedlings grow, so they can adapt to full sun. Cultural practices like irrigation fertilization and other practices should be performed as recommended for seedlings produced in trays or plastic bags (do Rêgo et al., 2016).

Before transplanting hardening of the seedlings is conducted for 7-10 days with gradual reduction of applied water and increasing full light sun. When the seedlings developed six to eight leaves transplanting is performed, which is about high of 10-15 cm occurs approximately 50-60 days after sowing for most species of pepper. Required spacing for planting is ranging from 1.20 m to 1.50 m between rows and 0.70 to 1.00 m between plants applied mostly for semi-evergreen shrubs with cycles greater than 12 months. So, the spacing varies depending on the cultivar, while narrow spacing retains more moisture and decreases incidence of pests but facilitates the attack of disease, cultivation and harvesting.

Fertilizer requirements for the cultivation of pepper must be based on the chemical analysis of soil, irrigation method and expected productivity by considering the amount of nutrients extracted by the crop. The recommended fertilizer rate varies depending on different authors due to different factors including growing condition (do Rêgo et al., 2016). To calculate the correct nutrient management different aspects, need to be available and taken into consideration. Nutrient withdrawal figures, fertilizer used before on the specific area intended to be planted, soil type, soil analyses, soil acidity, quality of irrigation water and micro elements. Based on this, the ideal soil analyses or soil status for sweet and hot pepper production should be PH : 5.6 – 6.8, P: 30 – 60 mg/Kg, K: 100 – 250 mg/Kg, Ca: 300 – 2000 mg/Kg, Mg: 120 – 300 mg/Kg and Na: 10 – 50 mg/Kg (Starke Ayres, 2019). It is common to use a land that had 4 years crop rotation made on as an organic plot and 4 kg/m^2 of sheep manure in the case of organic farming (Ribes-Moya et al., 2020).

1.4.3 Irrigation

The total water requirement depends on the type of pepper and the duration of development cycle, and it ranges from 500 to 800 mm and may exceed up to 1000 mm for long cycle cultivars. Specifically daily requirement of 3 to 10 mm/day in peak demand of the crop also reported. Watering should be daily or every three days depending on the texture from transplanting until the recovery of growth lasts about a week. It was reported that , fruiting from fill bloom until the beginning of fruit maturation is the most critical period regarding the deficiency of water, especially during the full flowering and fruit set stages (Freitas et al., 2008).

The way of application is also crucial in addition to water supply and amount for the successive growth of the crop. Among different ways of application, sprinkler is feasible for in various types of soils and topography as well as lower cost compared to drip irrigation. But, high incidence of foliar diseases, washing of pesticides and high canopy humidity are the main problems in sprinkler irrigation (Freitas et al., 2008). The most cost-effective irrigation system is surface irrigation by rows commonly used by small producers and have an advantage for reducing foliar diseases. Even if it is not suitable for high permeability sandy soils with steep slope. Currently, drip irrigation in a localized manner in the root zone, conservation of water and

energy and drip fertilization make an attractive system for cultivation of pepper (do Rêgo et al., 2016).

1.4.4 Weed, Pest, and Disease Management

Weed management in pepper can be achieved through all cultural practices that encourage rapid establishment of the pepper crop with good soil preparation, fertilization, proper spacing, directed irrigation, use of healthy and vigorous seedlings and crop rotation. Intercropping, with mulching at early stages of flowering (provide nutrients, especially nitrogen) are also another weed management option. Weed species of annual and perennial grown from seed can be controlled mechanically with rototillers, at the right time to ensure efficient control. Perennial species like *Camelina spp.* that propagate vegetative way require more attention because they can be favored by mechanical cultivation (do Rêgo et al., 2016).

Insects and mites associated with pepper can cause direct through the roots, stems, flowers, and fruits such as whitefly, sweet pepper fly, caterpillar weevils and others. Mainly aphids and thrips are the main reasons for indirect damage through transmitting viruses acting as a vector. Preventive control measures from planting and additional pest control measures must be considered (do Rêgo et al., 2016).

Pepper crop may be affected by fungal, bacterial, or viral diseases. Among Fungal etiology disease include: *Cercospora leaf spot, Powdery mildew, phytophtora wilt* and *anthracnose* can be mentioned. From disease of bacterial etiology highlight are the leaf spot and bacterial wilt. Viral disease of pepper includes potyvirus (*PVY/PepYMV)*, *Tospovirus ((TSWV, GRSV and TCSV*), TMV, and Begomovirus commonly transmitted by white fly (*Bemisia tabaci*). For the management of virus affecting peppers, it is advisable to produce or acquire seedlings grown in greenhouse from insect proof cages and away from the producing field, eliminate weeds around the crop field and destroy crop residues after harvest (do Rêgo et al., 2016).

1.4.5 Harvest and Post-Harvest

The harvesting period and crop cycle are directly affected by weather conditions, incidence of pest and disease and cultural practices such as fertilization, irrigation, and adoption of phytosanitary control measures. Generally, the first harvest of ripe fruits starts at 90 days after sowing for earlier varieties and 120 days for late mature peppers. They are harvested by hand, tearing up the fruits of plants with or without stems, depending on the type of pepper and the target market (do Rêgo et al., 2016).

For commercialization, different packaging according to the size and type of fruit, region and market demand are used. Plastic box or wooden type box containing 12-15 kg commonly used for the packaging of bigger fruits and smaller pepper fruits can be packed in carton boxes of 1-2 kg and plastic bags from 1 to 10 kg. All cultivated species of pepper behave non climacteric nature, not in need of ethylene production after they harvest. They require immediate reduction of temperature and wrapping with plastic film to avoid dehydration and shrinkage. Nonetheless of the domesticated species of hot peppers, the majority of fruits are susceptible to develop chilling symptoms when stored below 10 ℃ of temperature, which can be reduced by wrapping the fruits with plastic film (do Rêgo et al., 2016).

1.5 Spanish Pepper Landraces

Landraces, wild ancestors, and related species are important reservoirs of genetic variation that have not been exploited in breeding programs. In Spain, as other parts of Southern Europe, the versatility of agro-climatic regions and the heterogeneity of the land favored the survival in cultivation of a large number of specifically adapted landraces very diverse phenotypically (González-Pérez et al., 2014). Spain became a source of diversity for *C. annuum* due to the large heterogeneity in its agro-climatic conditions and its various culinary use of peppers, which give rise to a plethora of ecotypes in all the regions of the peninsula and islands (Ribes-Moya et al., 2020). Local varieties and ecotypes encompass a wide genetic diversity and evolved in breed under low input conditions, like those of the current organic agriculture. The previous taste of landraces made them highly demanded by consumers. Moreover, another highly demanded trait

from consumers is the wide diversity encompassed by traditional cultivars gives the opportunity to select high added value materials on the basis of their composition (Ribes-Moya et al., 2018).

In Spain, there are centers and institutions on a larger scale their own germplasm bank, which house collections of *C. annuum*, Spanish and foreign and other species. They are recognized nationally and internationally. The most relevant repositories correspond to the germplasm bank of the Institute for the Conservation and Improvement of Valencia Agrodiversity (COMAV-UPV, Valencia, BGV codes) and the Horticultural Germplasm Bank of the center for Agrifood research and Technology Aragon (CITA, Zaragoza, code BGHZ). Likewise, the Center for Plant Genetic Resources of the INIA (CRF-INIA, Madrid, with BGE code) is the largest repository in the country and preserves replicas of most of the accessions. A group of ecotypes whose historical, cultural, and agricultural roots have been recognized through PDOs or PGIs by the EU or other local quality seals. Varieties such as Piquillo de Lodosa, Pimiento del Bierzo, Pimiento Riojano, Morrón de Fresno y Benavente, Bola de Murcia, cornicabras de la Vera or the group of Galician peppers, among others, are the result of a process of selection and traditional conservation. These materials contain a great potential to adapt to conditions of sustainable agriculture, low inputs and adaptation to climate change (Ruiz et al., 2016).

1.6 Interests in the Improvement of Pepper Cultivation

Different breeding programs have been conducted in the past, as well as in recent years, on various types of *Capsicum annuum* species. The main goals of this program are to improve yield and fruit quality, including maturity and resistance to different biotic and abiotic stress conditions, through both conventional and non-conventional methods (Mohan Rao & Anilkumar, 2020).

The primary focus of quality improvement programs is on the selection and development of pepper breeding lines with biochemical compounds that have high antioxidant properties, ascorbic acid (vitamin C), phenolics and flavonoids (Sharma et al., 2020). These secondary metabolites found in peppers have been shown to have beneficial effect in fighting against cancer and cardiovascular diseases, as well as slowing down aging and atherosclerosis, in recent decades (Fratianni et al., 2020). In addition to the genetic factor agronomic management,

maturity stage and environmental factors can also influence these quality attributes (Jawad et al., 2013).

1.6.1 Phenolic Compounds

Phenolic compounds are secondary metabolites that are synthesized by plants because of plant response for biotic and abiotic stress conditions (Disease infection, wounding, water stress, cold stress, high visible light) (Materska & Perucka, 2005). Human body cannot produce this chemical compound unless they are taken through a diet. Secondary metabolites play an advantage for human nutrition because they are major determinants of fruit and vegetables quality. Phenylpropanoids, or phenolic compounds, terpenoids, alkaloids, and sulfuric compounds are the major distinguished groups of secondary metabolites and from these phenolic compounds, flavonoids have been shown to possess beneficial properties for human health and disease prevention in addition to their contribution for the taste, flavor, and aroma of a fruit (Lemos et al., 2019).

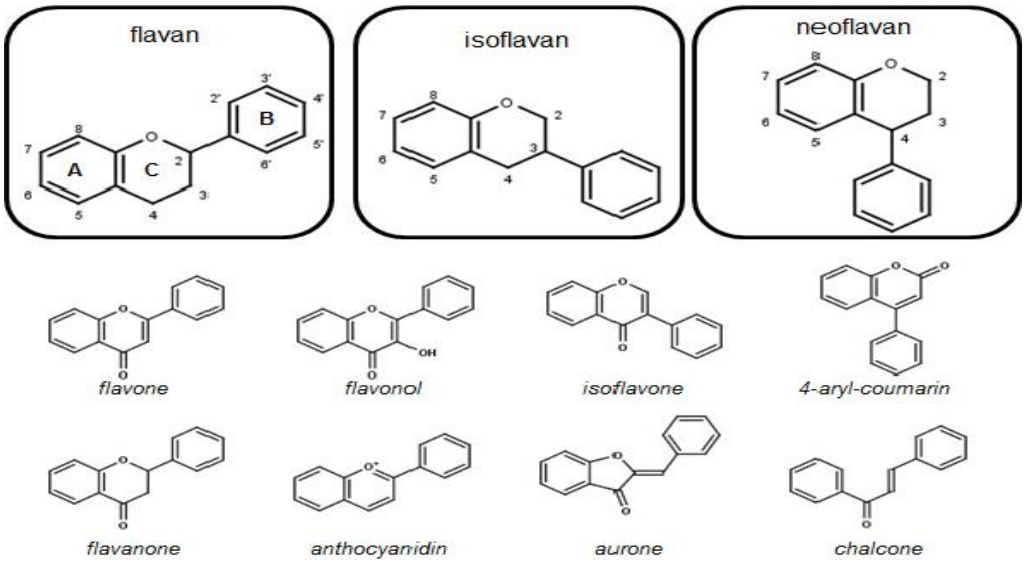


Figure 5. Structure of the structural backbones of the main flavonoid groups (flavan, isoflavan and neoflavan) and of relevant flavonoid classes (Baenas et al., 2019).

Around 80% of flavonoids are found in the most common classes that are flavones, flavonols, flavanones, catechins, isoflavones and anthocyanidins (Pinheiro et al., 2012). The phenylpropanoid pathway is the source for phenolic antioxidants and in its initial step phenylalanine transforms into cinnamic acids. Condensation of cinnamic acids with three malonyl-CoA groups produce flavonoids and further can undergo enzymatic esterification with plant sugars (Asnin & Park, 2015).Various epidemiological studies revealed the association between uptake of phenolic acids and flavonoids and the reduction in the risk for coronary disorders, diabetes, cancer, osteoporosis, and neurodegenerative diseases (Baenas et al., 2019).

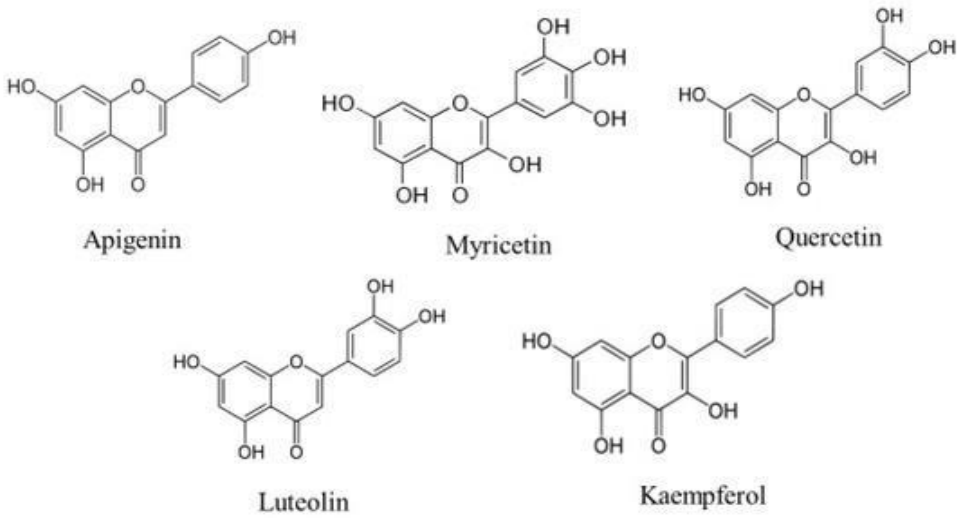


Figure 6. Molecular structure of the main dietary flavonols and flavones (Hernández-Pérez et al., 2020)

Flavones and flavanols have antioxidant effects and free radical scavenging activity in foods, and have significant vitamin c sparing activity, from this myricetin is one of the most active. Flavanol glycosides (quercetin) predominate in vegetables, glycosides of kaempferol, luteolin and apigenin are also present (Do Socorro Chagas et al., 2022). Most flavonoids in pepper are flavonoid aglycones that are quercetin and luteolin and the concentration of this antioxidants increase with maturity (Hernández-Pérez et al., 2020).

It has been reported that fully ripe stage fruits of hot chili peppers have 1.2-fold higher total phenolic content that the full green stage. Similarly, 90% red stage showed 5.3-fold total flavonoids compared to 100% green stage, but its content tends to decrease in the over ripe stage

(Shiau et al., 2023). There are several factors such as variety, ripening stage and growing system affects the level of phenolic compounds (Ribes-Moya et al., 2020).

1.6.2 Carotenoids

Carotenoids are naturally occurring bright color pigments that are important physiological functions, responsible for the quenching of light and protect cells from damages caused by light and superoxide radicles in all photosynthetic organisms such as algae, cyanobacteria, and plants (Swapnil et al., 2021). They are synthesized also in non-photosynthetic organisms like bacteria and fungi. They are not synthesized by animals, so their presence is through dietary intake. They play a special role in protecting tissues from light and oxygen for plant and animal health, apart from their color pigmentation. Commercially they are exploited as food colorants and feed additives and used in pharmaceutical, nutraceutical and cosmeceutical products (Arimboor et al., 2015).

Carotenoids are water insoluble lipophilic metabolites containing a chromophore that comprise a long polyene central chain of conjugated double bonds (maximal absorbance wavelength 400-500 mm), that is responsible for yellow to orange characteristic and reddish colors of these compounds (Liaaen-Jensen & Andrewes, 1972). Two types of carotenoids are found in vascular plants, the unoxygenated carotenoid referred as carotenes (e.g., β-carotene and lycopene) and the oxygenated carotenoids are named as xanthophylls (such as lutein and zeaxanthin). The property and function of this molecules primary depend on its chemical structure, carotenoids are mostly 40-carbon terpenoids with eight isoprenoid units as their basic structural unit, joined in a specific manner to facilitate the position of nonterminal methyl groups and two central methyl groups in a 1,5-position and 1,6-position, respectively (Swapnil et al., 2021).

Prominent physiological importance has been proposed for carotenoids. β-carotene beside with α-carotene and β- cryptoxanthin are sources of provitamin A. Studies describe the inverse relationship between intake of high carotenoid containing diet and human chronic disease incidents.

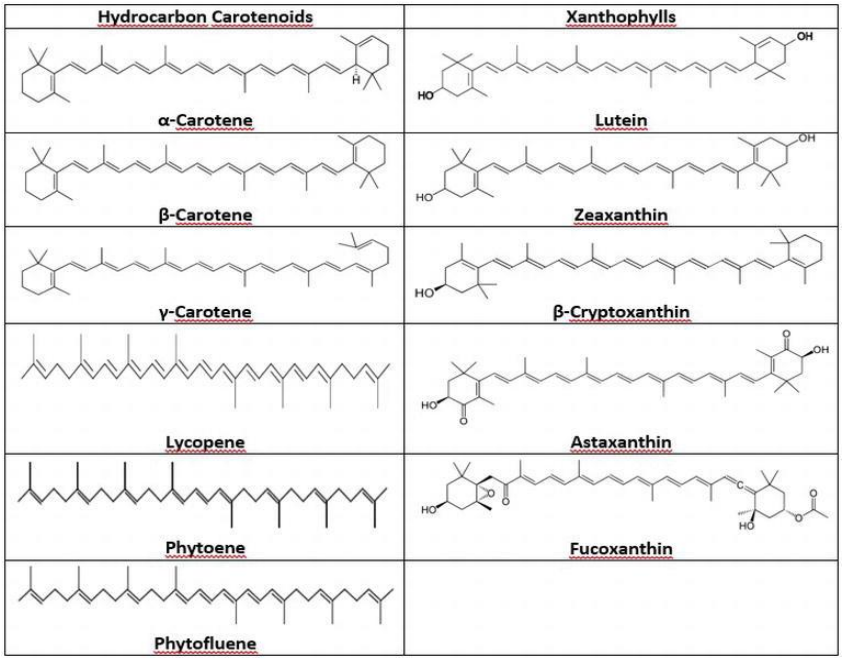


Figure 7. Classification of carotenoids according to their structures (Merhan, 2017).

They also play a role in reducing oxidative stress, inhibit cancer cells, and offer protection from cardiovascular diseases, macular degeneration, and contract (Arimboor et al., 2015). Pepper fruit is some of the ancient and most widely used food additives and its typical red color found in capsicum is derived from the capsanthin and capsorubin components that are exclusively synthesized and accumulated during fruit ripening in this genus (Ha et al., 2007). Raw peppers are main sources of carotenoids, due to genetic differences and degree of maturation, production practice and processing conditions their composition and content may vary. The amount of total carotenoids present in mature pepper fruits can vary significantly, with levels ranging from 0.69 to 30 mg per gram of dry weight or 15 to 320 mg per 100 grams of fresh weight (Baenas et al., 2019).

1.6.3 Sugars

Sugars are assimilating that participate in the photosynthetic fixation of carbon in plants, due to this universal role they found in all parts of pepper plant. Fructose, glucose, and sucrose are the major sugars, in the fruit pericarp nonstructural sugars are accumulated in the fruit pericarp in

10-fold higher amount than in seed or leaves. Sugars are primary compounds responsible for the sweet taste in capsicum. Their amount may vary among varieties or cultivars and ripening process (Asnin & Park, 2015).

Pepper has high vitamin C, food containing vitamin C commonly characterized by high carbohydrate content and a high sugar concentration. Fructose and glucose levels are almost similar, while sucrose concentration is minimal decreasing with maturation to non-detectable levels in red fruits (Navarro et al., 2006). Studies in varietal type and ripening stage indicate that, several varieties sugar content showed significant result reaching to those of tomatoes or carrots at fully ripe stage, which is 30-50 g/100 g of fresh weight (Guijarro-Real et al., 2023). *C. annuum* has lowest total sugar (448 g/ kg DW) compared to *C. chinense* × *C. frutescens* fruits (747.1 g/kg DW). Fructose has been the most abundant sugar followed by glucose and sucrose in both pericarp and placenta of chili fruit, ripeness increases the sugar content, but it is not correlated with the color (Zamljen et al., 2021).

1.6.4 Ascorbic Acid

Capsicum is considered an excellent source of vitamin C, E, and precursors of vitamin A, that is called provitamin A. Both vitamin C and (L-ascorbic acid) and vitamin E (tocopherols) are appreciated for their antioxidant properties and synthesized by plants as protectants against reactive oxygen species, which are generated in photosynthesis and respiratory process.

L-Ascorbic acid (AA) and its oxidation product L- dehydroascorbic (DHAA) acid are the two existing forms of vitamin C. According to some authors DHAA are not a vitamer as it is existed in a small amount of the total vitamin C in capsicum and does not contribute for the antioxidant capacity of the vitamin (Dhatt et al., 2020), but other authors do because it can be converted metabolically to AA (Howard & Wildman, 2007).

Ascorbic acid ($AscH_2$, vitamin C) is water soluble ketolactone two ionizable hydroxyl groups, two pKa's, pK1 is 4.2 and pK2 is 11.6; thus, the ascorbate monoanion, AscH-, is the dominant form at physiological PH. Ascorbate maintains Fe2+ of collagen hydroxylases in an active state; it plays a pivotal role in collagen synthesis; parallel reactions with a variety of dioxygenases

affect the expression of a wide array of genes. Its mechanism of action in full potential of pharmacological ascorbate in cancer treatment (HOWARD et al., 1994).

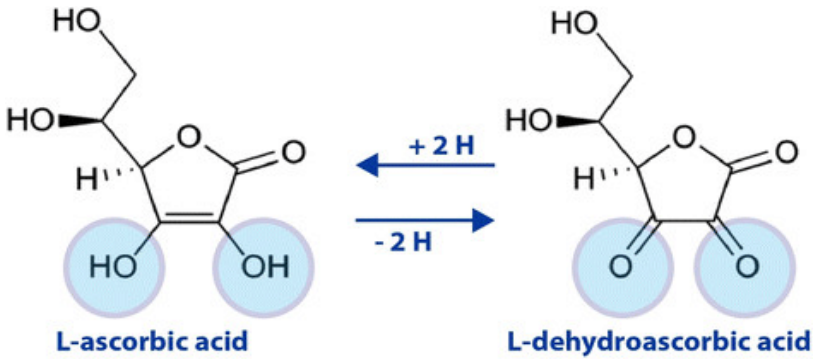


Figure 8. The oxidation-reduction (redox) reaction of vitamin C, molecular forms in equilibrium (Lemos et al., 2019).

A study on fresh pepper cultivars examined that mature red peppers have 95% more ascorbic acid than mature green, ranged from 76 mg/100g for mature green to 243 mg/100g for mature red peppers (Dubick & Omaye, 2016).

1.7 Maturity and Pepper Fruit Quality

One of the main factor that determines the compositional quality of fruit and vegetables are maturity (Lee & Kader, 2000). Phytochemical alterations that occur during ripening/maturation and resultant effect on antioxidant activity are important dietary considerations that may affect the consumption of different pepper types (Du et al., 2012). Studies suggested that there has been significant difference on the content of bioactive compounds on different pepper varieties, bell pepper have high content of bioactive compounds compared to other chili varieties, also maturity is the main factor for the variation of this compounds (Shaha et al., 2013).

The parts of the pepper fruit such as pericarp and placenta, the time of harvest are also crucial factors that determines changes in capsaicinoid content (Vázquez-Espinosa et al., 2023). Consumer preference and use in pharmaceutical industries is the main determinant to distinguish

and recommend different types of pepper varieties. It has been demonstrated that for hot chili varieties 80% green recommended for pharmaceutical industry purpose as its high capsaicinoid content, antioxidant property and acceptable phenolic and flavonoid content, while 100% red stage is recommended for fresh or cooked dishes (Shiau et al., 2023).

1.8 Organic Farming

Organic farming is an agricultural production system that follows the four principles: i) health for human, animal, plant, and land, ii) maintaining biodiversity and ecological balance, iii) providing fairness in the environment and life possibilities, and iv) protecting the health of the world's present and future generation. These are the International Federation of Organic Agriculture Movements (IFOAM's) principles which are Health, Ecology, Fairness and Care (IFOAM,2016). Organic farming is as system designed to minimize the need for external inputs of nutrients, crop protection, aids, and preventive medicines. This system produces food without use of chemical or synthetic fertilizers, pesticides and herbicides, since it relies entirely on bio-fertilizers, natural pathogens control and thereby benefits the sick planet (Lone & Rashid, 2023). It has been demonstrated that organic farming has a favorable impact on environmental and ecological setup simultaneously addressing varied economic and social aspects as well (Lone & Rashid, 2023). Various studies revealed that organic crops are better source of nutrients, give higher animal immunity, increased disease resistance to plants, 50% less mycotoxins in crops and persistent shelf life. In OF system plants generate more secondary metabolites, higher micronutrients, and more coupled fatty acids for better human health, including lower incidences of noncommunicable disease (Siddique et al., 2014).

1.8.1 Organic Farming in the Globe

The practice of organic farming has been adopted in 191 countries across the world. At least 3.7 million farmers manage over 76 million hectares of agricultural land using organic methods. The global market for organic products (food and drinks) reached almost 125 billion euros in 2021. Oceania was the most organic agricultural land region with 36.0 million hectares followed by

Europe with 17.8 million hectares, Latin America (9.9 million hectares), Asia (6.5 million hectares), North America (3.5 million hectares) and Africa with 2.7 million hectares (FiBL, 2023).

Figure 9. World Distribution of organic agricultural land by region 2021 (FiBL, 2023).

The country with the most agricultural land was Australia, it accounts 97% of grazing farmlands (Figure 10). Argentina is the second country followed by the European country France, including the remaining ten countries with the largest agricultural areas have a combined total of 59.6 million hectares and constitute almost 80% of the worlds organic agricultural land (FiBL, 2023).

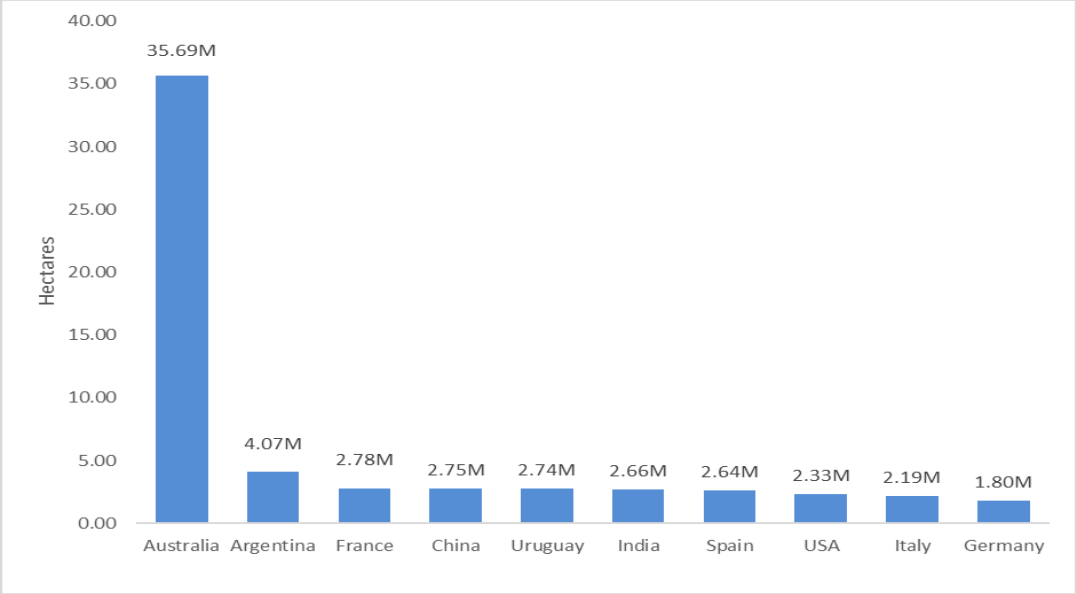


Figure 10. The ten countries with the largest areas of organic agricultural land 2021 (FiBL,2023).

1.8.2 Regulation of Organic Farming

According to the latest data collected by IFOAM _organics international 2022, the new Eu organic regulation has started to apply on 1 January 2022. The basic regulation for organic (EU) 2018/848 was published in June 2018, while the secondary legislation has been developed in the coming 2.5 years. There are 74 that have fully implemented regulations and a total of 21 countries that have not fully implemented organic agriculture with 15 drafting legislations. European union, New Zealand and the USA have undergoing significant revisions (Willer et al., 2023).

Almost 18 million hectares of farmland in Europe were managed organically in 2021. More than half of the European countries organic farmland is under four countries; France (2.8 million hectares), followed by Spain (2.6 million hectares), Italy (2.2 million hectares) and Germany (1.8 million hectares) (FiBL, 2023).

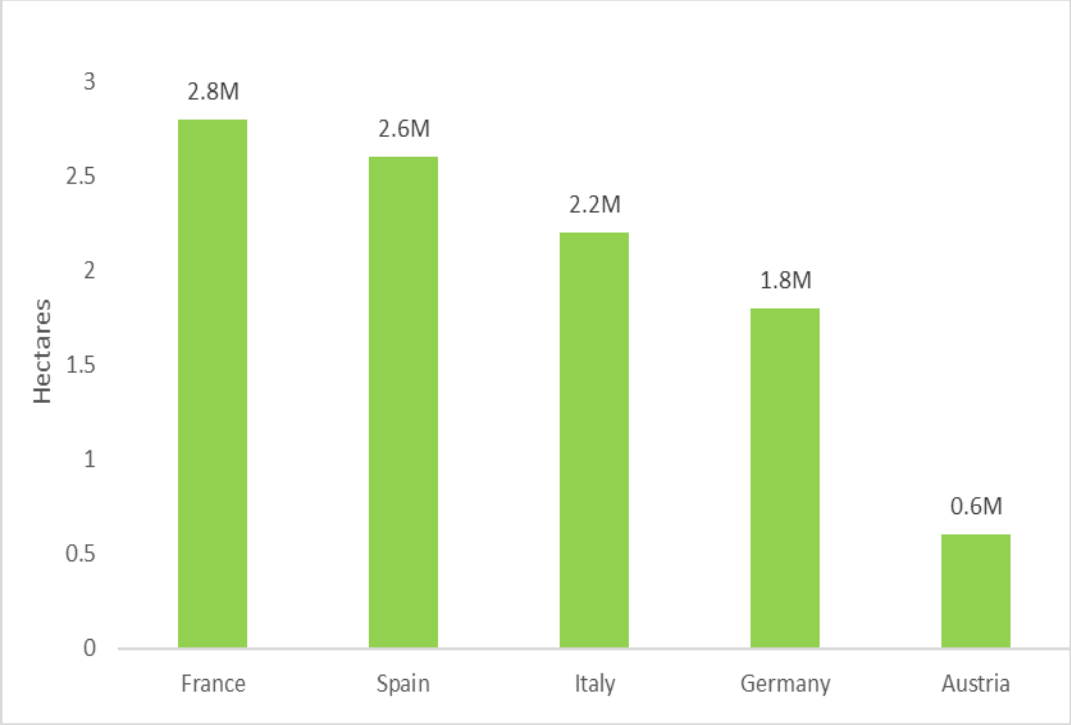


Figure 11. Top 5 European countries with the largest organic farming acreage (FiBL, 2023).

1.8.1 Organic Farming Effect on Fruit Quality and Yield

Environmental stress condition in organic farming plays a vital role for plants to produce secondary metabolites, this biologically active phytochemicals in plant foods offer health benefits against several chronic diseases (Young et al., 2005). Consumers interest for organic foods has been attributed to the growing demand for food free from pesticides and chemical residues (Thangaraja, 2022). Findings pointed out, organic fruits contained significantly more dry matter, vitamin C, total carotenoids, β-carotene, α-carotene, *cis*-β-carotene, total phenolic acids and flavonoids (quercetin D-glucoside, quercetin and kaempferol) compared with conventional fruits (Hallmann & Rembial kowska, 2012). In addition to the growing system there are other factors such as variety, maturity/ripening stage determines the level of this bioactive compounds in fruits and vegetables. Studies on growing system, including variety and ripening stage effect on collection of pepper (*Capsicum spp*) has been reported significant variation between conventional cultivation and organic farming in total flavonoid content and the ripening process increased the level of flavonoids under organic cultivation (Ribes-Moya et al., 2020).

For the success of organic farming, high and good quality yield is required. Overall, organic farming produces lower yield than conventional, vegetables yield 30% lower than conventional condition. The main reason for this lower yielding is insufficient fertilizer availability, particularly nitrogen approach. In organic farming soil organic matter is used as a source of nitrogen and increasing soil chemical, physical and biological fertility is an overall objective of organic farming (Szafirowska & Elkner, 2009).

Previous works conducted by Mac Rae et al. (2006) during the first years the yield of organic vegetables was 40% lower than the conventional method. After the built up of the soil fertility and biodiversity, 5-10 years after conversion, the differences significantly diminished. Other findings also suggested organic cultivation in vegetables positively affect growth by 43%, yield by 59% and 65% better nutritional value. While 86% of results showed a reduction in nitrate levels in organic cultivation. If the appropriate technology is used the organic cultivation of vegetables is not so time and money-consuming and the trend is that, with organic cultivation, vegetables with better quality and better nutritional value with no pesticide residues can be produced (Lone & Rashid, 2023). Well managed organic farming specifically on vegetables can

provide food security and healthy diet for humans, while being less harmful to the environment and more efficient in natural resource use. It is evident that organic farming is a sustainable approach impact on environmental and ecological setup simultaneously addressing varied economic and social aspects (Lone & Rashid, 2023).

1.9 Agroforestry

According to Lundgren (1982, as cited in Murthy, 2017), agroforestry is a collective name for land use systems in which woody perennials (trees, shrubs, etc.) are grown in association with herbaceous plants (crops, pastures) or livestock in a spatial arrangement, a rotation or both for the benefit of ecological and economical interactions between the trees and other components of the systems. In this system tree and no-tree species intentionally designed and managed to increase positive interactions and it encompasses a wide range of practices like contour farming, intercropping, established shelterbelts, riparian zones/buffer strips etc. Trees are an important part of the natural ecosystems and their presence in the system of agriculture provides a range of benefits to the soil environment (Murthy et al., 2017). In agroforestry system wood plants contribute to modify the microclimate by reducing evapotranspiration and moderating extremes in soil temperature and daily photosynthetically active radiation. In this micro environment, light intensity have an impact on plant secondary metabolites (Re et al., 2019).

In agroforestry system, allelopathy plays an important role leading to a wide range array of interactions between crop-crop and tree-crops. Allelopathy can be defined as any direct or indirect positive or negative effect of one plant on the other through the release of chemicals in to the environment (Joshi et al., 2016). Soil microbes play a great role in determining such interactions as they not only alter the nature of allelopathy interactions but also modify the expression of allelochemicals. Allelopathic crops also play a role in reducing incidence of soil born disease through crop residues releasing inhibitory substances. Studies also pointed out the negative impact of allelochemicals in suppressing rate of germination, plant height, number of leaves per plant and yield, when there is high concentration allelochemical extract (Devi, 2017). In general, allelochemicals from crops as well as from other plants can be utilized for the purpose of managing weeds, pathogens, disease, insect, and nematodes under integrated pest and

weed management programs in sustainable agriculture. These natural products have short environmental life, low environmental impact and mammalian toxicity and are more site specific and target oriented (Joshi et al., 2016).

Given the aforementioned considerations, there is a need to expand the background knowledge regarding the impact of organic agroforestry farming on the essential phytochemical compounds present in widely adapted pepper landraces. This study aims to provide a deeper understanding of how organic agroforestry conditions can influence the yield and quality attributes, particularly focusing on secondary metabolites such as phenolic compounds and specific phytochemicals, across different maturity stages of various landrace varieties.

2. OBJECTIVES

To assess the main fruit phenotypic traits of Spanish local pepper varieties under organic agroforestry cultivation.

To evaluate the nutritional quality of *capsicum* fruits of important bioactive compounds such as, carotenoids, flavonoids, phenolics, ascorbic acid and sugars in response to organic agroforestry farming.

To examine how differences in maturity affect the levels of those key bioactive compounds.

To analyze the combined effect of variety and maturity in response to organic farming.

To identify possible correlations between compounds of interest.

3. MATERIALS AND METHODS

3.1 Plant Material

In this experiment, five local Spanish pepper varieties belonging to the species *Capsicum annum L.* have been evaluated. These varieties were Guindilla, Bierzo, Largo de Reus, Blanco de Villena, and Bola as mentioned in Table 3. They were grown under organic farming to evaluate their agronomic and nutritional quality variations. Fruits were harvested at green and red maturity stages, to analyze the effect of maturity on the nutritional aspects specifically, flavonoids, carotenoids, phenolics, sugars, and ascorbic acid contents of each tested variety.

Table 3.List of varieties used with local name, origin, and fruit traits.

Variety	**Origin**	**Green ripe color**	**Fully ripe color**	**Mesocarp**	**Shape**	**Length/ width (mm)**	**Weight (g)**
Bierzo	Bierzo PGI, Carracedelo, León	Green	Red	Thick, fleshy	Blocky	110/80	140
Bola	Totana, pimento PDO de Murcia	Green	Deep red	Thin, high dry matter	Almost round	40/40	23
Guindilla	Arkaute, Álava	Green	Red	Thin, high dry matter	Elongate	140/13	9.5
Largo de Reus	Reus, Tarragona	Green	Red	Thick, fleshy	Blocky	150/90	180
Blanco de Villena	Villena, Alacant	White	Red	Medium thick	Triangular	120/60	108

3.2 Growing Conditions

The plant materials for this study were grown in the field under specific environmental conditions of agroforestry in Portugal. The field was located on industrial zone, at Mendes Gonçalves, Golegã, with coordinates 39º 24’ 43.6” N and 8º 29’ 43.6” W. The soil had medium

texture with acidic to neutral PH. The plants were cultivated during spring summer growing season in 2022, within an average temperature range of 18℃- 32℃ (Figure 12).

The pepper seedlings were transplanted into the field plot when they reached at 4 true leave stage in the spring season. Prior to planting, the soil was prepared through tilling and aeration. Additionally, sheep manure was applied at a rate of 4 kg per meter square. Throughout the growing period, various organic agronomic management activities were performed, including manual weeding.

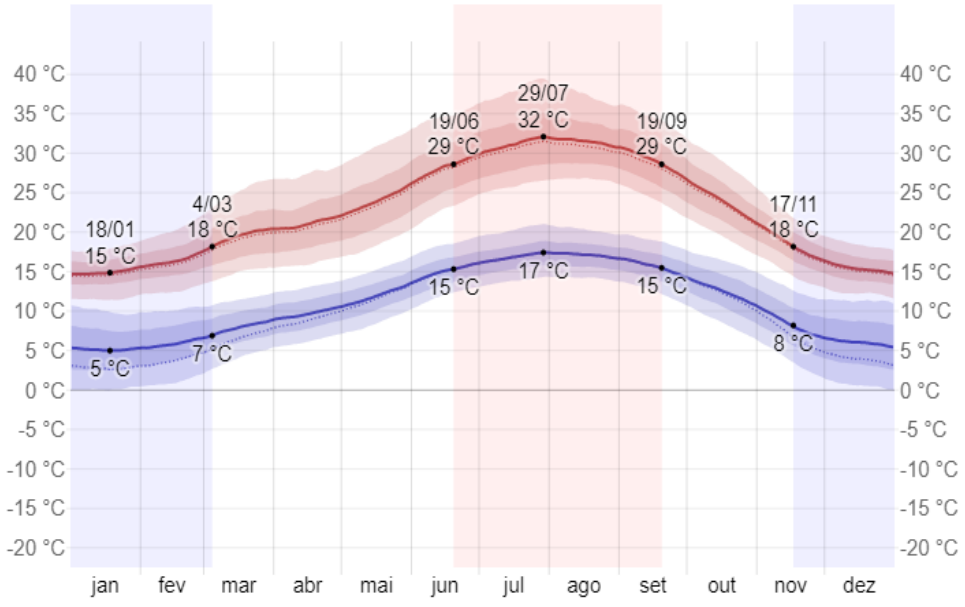


Figure 12.Climatic data of field located at Mendes Gonçalves during 2022.

The experiment took place in an agroforestry system managed under organic farming practices. The agroforestry condition consisted of a plot with rows of a mixture of tree species, namely Ajuga (*Ajuga reptans*), Gazania (*Gazania rigens*), Murta (*Myrtle, Myrtus communis*), Apple tree (*Malus domestica*), Poplar tree (*Populus sp.*), Blackberry bush (*Rubus fruticosus*), Pepper tree (*Schinus molle*), Fig tree (*Ficus carica*), Elder tree (*Sambucus nigra*), Eucalyptus (*Eucalyptus globulus*) and Grapevine (*Vitis vinifera*) (Figure 13).

3.3 Experimental Design

The experimental design employed in this study was a randomized complete block design. Three replications were included to ensure statistical robustness and account for potential variability within the experiment. The seedlings were transplanted with a specific spacing of 0.7 and 1.0 m between plants and rows respectively. It is also important to note that agronomic data were collected only from the middle representative plants to minimize potential border effects.

Figure 13. Agroforestry experimental field at Mendes Gonçalves Portugal at the vegetative stage of the tested varieties.

Harvesting was carried out at two different commercial maturity stages: i) green ripe but reaching final size and firm fruit and ii) fully ripe with complete carotenoid coverage (final size and firm fruit) (Figure 14). These stages were determined based on the experience and expertise of the technician involved in the study, as well as commercial considerations.

Figure 14.Harvested fruits of tested varieties at green ripe and fully ripe state. (a) Largo de Reus (b) Blanco *de Villena (c) Bierzo (d) Bola and (e) Guindilla.*

3.4 Phenotypic and Agronomic Parameters

Phenotypic and agronomic data were recorded based on the descriptors of *Capsicum* spp (Ipgri, 1995). These descriptors provide a comprehensive overview of the physical traits and characteristics of the peppers. Qualitative characters were recorded based on visual observation while quantitative characters were measured and recorded by using a measuring meter and weighting balance. For each variety ten randomly selected fruit were used for quantitative data measurement.

3.4.1 Qualitative Fruit Characters

The qualitative fruit characters evaluated were fruit color at intermediate stage, fruit color at mature stage, fruit surface, fruit shape, fruit shape at pedicel attachment, fruit shape at blossom and fruit cross-sectional corrugation.

Fruit colour at intermediate stage was determined on fruits just before the ripening stage based on the six capsicum fruit colour descriptors at intermediate stage such as, white, yellow, green, orange, purple and deep purple. Fruit colour at mature stage was also examined by looking at each sample fruit's colour based on the thirteen fruit colour descriptors at mature stage. These colours include white, lemon-yellow, pale orange-yellow, pale orange, orange light red, red, dark red, purple, brown, and black. Based on the three fruit surface descriptors (smooth, semi wrinkled and wrinkled) each variety representative sample fruits, fruit surface was determined visually.

Fruit shapes were examined based on the six capsicum fruit shape descriptors which are: elongate, almost round, triangular, campanulate, blocky and other as a specification (Figure 15).

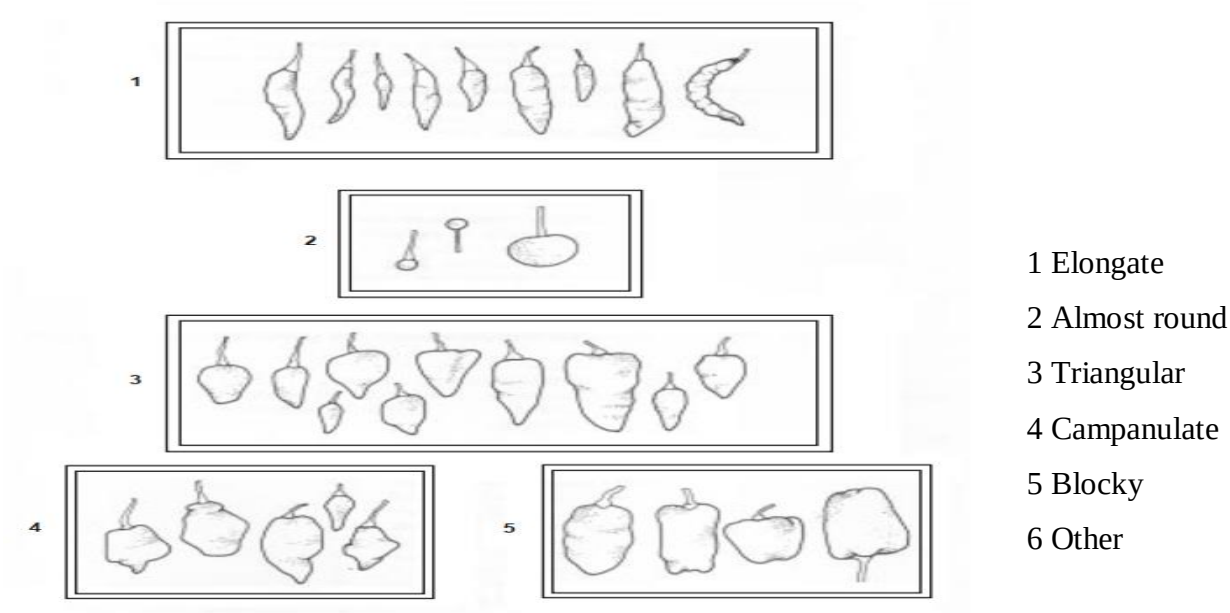
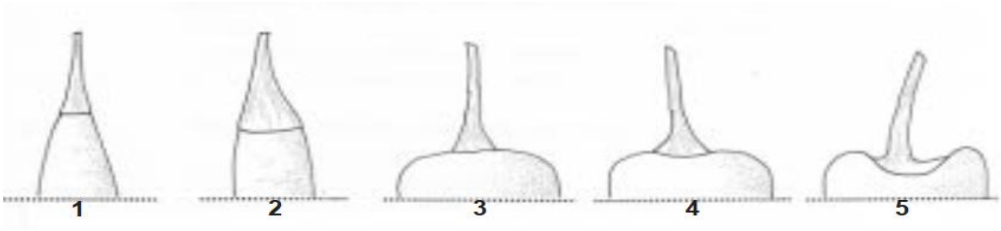


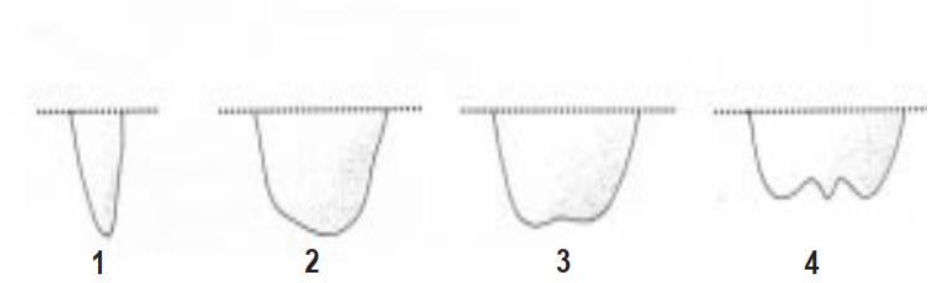
Figure 15. Capsicum fruit shape descriptors (IPGRI, 1995)

Fruit shape at pedicel attachment were determined using five different shape descriptors as illustrated in the figure 16. Fruit shape at blossom end were examined based on four different types of shape descriptors on an average of 10 fruits (Figure 17). An average of 10 fruits (1/3 from pedicel end) were used to evaluate fruit cross-sectional corrugation as shown in figure 18.



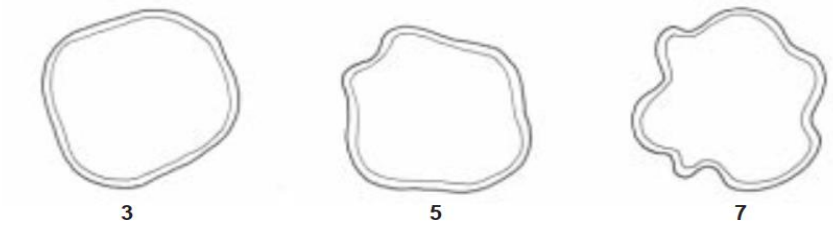
1 Acute
2 Obtuse
3 Truncate
4 Cordate
5 Blocky

Figure 16. Capsicum fruit shape at pedicel attachment descriptor.



1 Pointed
2 Blunt
3 Sunken
4 Sunken and pointed
5 Other

Figure 17. Shape at blossom end descriptors.



3 Slightly corrugated
5 Intermediate
7 corrugated

Figure 18. Fruit cross-sectional corrugation descriptors.

3.4.2 Quantitative Fruit Characters

Fruit length, fruit diameter, fruit weight, pedicel length, number of loculus (seed compartments), were measured form 10 representative sampled fruits and its average were considered. In addition, fruit fresh weight, fruit dry weight (measured at a specific temperature range) and total productivity data were collected as a quantitative fruit trait parameter.

3.5 Sample Preparation for Bioactive Compound and Nutritional Quality Determination

Pepper fruits were harvested at two commercial maturity stages, green ripe and fully ripe (red), to evaluate their nutritional content variation. Fruits of two plants in middle rows for each variety by ripening stage were considered. For the nutritional quality analysis, mesocarp sample of each variety was lyophilized in a wizard 2.0 freeze drier (VirTis, Warminster, PA, USA) (Figure 19). The samples of each variety separated by maturity were milled through coffee grinder and powder samples were placed in sealed falcon tubes and stored in dry and dark conditions until the extractions and analyses of biochemical compounds were made (Figure 20).

Figure 19. Lyophilized samples of green ripe and fully ripe (red) pepper fruits.

Figure 20. Sample grinding and preparation through coffee miller.

Preserved powder samples were used for further analysis of carotenoids, phenolics, sugars, flavonoids, and ascorbic acid. All phenolics, sugars, ascorbic acid and flavonoids were estimated in both green ripe and fully ripe stage samples except for the carotenoid it was estimated only in the fully ripe samples due to their low/null content of carotenoids in green ripe fruits.

Thus, a total of 1288 samples were analyzed from both green ripe and fully ripe stage fruits: 750 samples of three repetitions for flavonoids and phenolic analysis, 400 samples of two repetitions for sugars and ascorbic acid, 138 samples of three repetitions for carotenoids only on fully ripe fruits (Figure 21).

Figure 21. Powdered pepper samples for nutritional quality analysis.

3.6 Analytical Methods

3.6.1 Carotenoid Analysis

Extraction of Carotenoids in Pepper Samples

The spectrometric method developed by Hornero-Méndez and Mínguez-Mosquera (2001) with some modification was used for the total carotenoid analysis. The method consists of the measurement of absorbance in the UV-visible region at two characteristic wavelengths. For multicomponent mixtures equation of Lambert-Beer law was applied by the most appropriate

wavelengths of 472 nm and 508 nm for the quantification of the red and yellow fractions in acetone (Hornero-Méndez & Minguez-Mosquera, 2001).

To make the extraction, 100 mg dry sample was extracted with 20 ml of acetone for 1 hour in automatic shaker (Figure 22). The resulting extract was then filtered by using paper filter and transferred to a 25 ml volumetric flask by using acetone to make up the mark. The spectrometric readings were conducted through BioTek EPOCH/2 microplate reader and 1cm optical path glass cuvettes (Figure 23). Acetone was used as a blank and reading of each sample extract was performed at the specified wavelength. Subsequently, the red, yellow, and total carotenoids were determined by using the formula provided (Hornero-Méndez & Minguez-Mosquera, 2001):

$$C_R = \frac{A508 \times 2144.0 - A472 \times 403.3}{270.9} (\mu g/mL) \qquad C_Y = \frac{A472 \times 1724.3 - A508 \times 2450.1}{270.9} (\mu g/mL)$$

$$C_T = C_R + C_Y$$

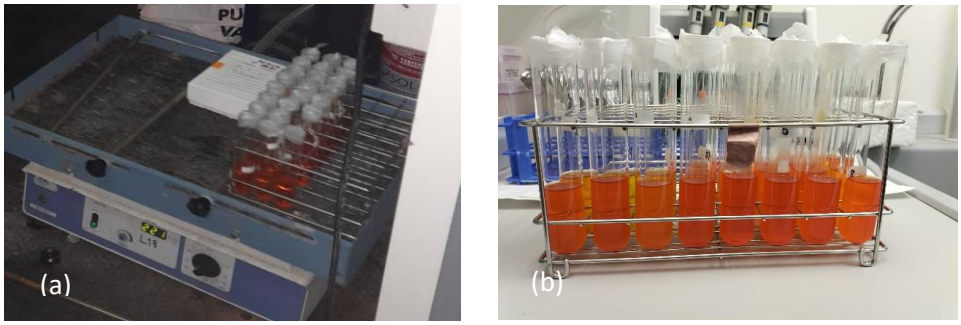


Figure 22. (a) Samples of pepper in the automatic shaker, (b) Extracted pepper samples for carotenoid determination.

Figure 23. Spectrometric reading through BioTek instruments (EPOCH2TSC Agilent Technologies, USA).

3.6.2 Total Phenolics Determination

Sample Extraction for Phenolics Determination

The extraction of phenolics was carried out by following the procedure described in Ainsworth & Gillespie (2007) with slight modifications. The method depends on Folin-Ciocalteu assay that relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/ phosphotungestic acid complexes to form blue complexes that are determined spectroscopically at approximately 760 nm (Singleton et al., 1999).

The subsample of 100 mg pepper powder was used for the extraction of phenolics. 10 mL of the extracting solution (80:20 Methanol/water v: v, with 0.1% butyl-hydroxy-toluene (BHT)) were added to the centrifuge tube containing powder samples. The samples were placed in automatic shaker without light at -4°C for 1 hour (Figure 24). The mixture was then centrifuged at 3500 rpm for 15 min at room temperature. 10 µL of each sample extract, standard calibration solutions and blanks were transferred to a 96-well microplate. Then 100 µL of diluted Folin_ Ciocalteu reagent (10 mL Folin Ciocalteu reagent and 90 mL of pure water) were added to each well. After 5 minutes in dark conditions, 100 µL of sodium carbonate solution (Na_2CO_3) of 60 g/L was added and left for 90 minutes without light (Figure 25). Finally, the absorbance of blue color was measured at 765 nm using a 96 well plate EPOCH/2 microplate reader (BioTek Instruments) (Figure 26). Gallic acid was used to create the calibration curve, and total phenolics were expressed as µg of gallic acid equivalent/g of pepper.

Figure 24. Extracted samples of pepper for phenolics determination.

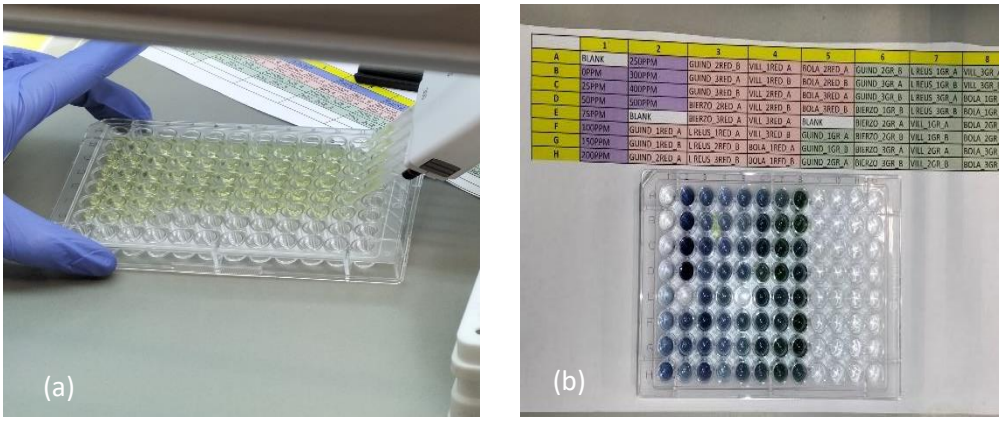


Figure 25. (a) Extracted sample with Folin_ ciocalteu reagent in a 96-well microplate (b) Reaction between the Folin-Ciocalteu reagent and Na2CO3.

Calibration Curve

112 mg of gallic acid with 97.5 % purity was diluted in 100 mL of water and used as a stock solution to prepare the standard calibration curve. Seven standard values (0 ppm, 15 ppm, 31 ppm, 62.5 ppm, 125 ppm, 250 ppm and 500 ppm) were used. Serial dilutions of ½ from the stock solution were used to obtain the calibration curve. All the absorbances were corrected with blank measures. Finally, a standard curve from A_{765} of the gallic acid standards was calculated. Total phenolics of each sample was determined as gallic acid equivalents by using the regression equation between the gallic acid standards and A_{765} (Figure 27).

Figure 26. Phenolic absorbance reading EPOCH2TSC microplate reader (Agilent Technologies, USA).

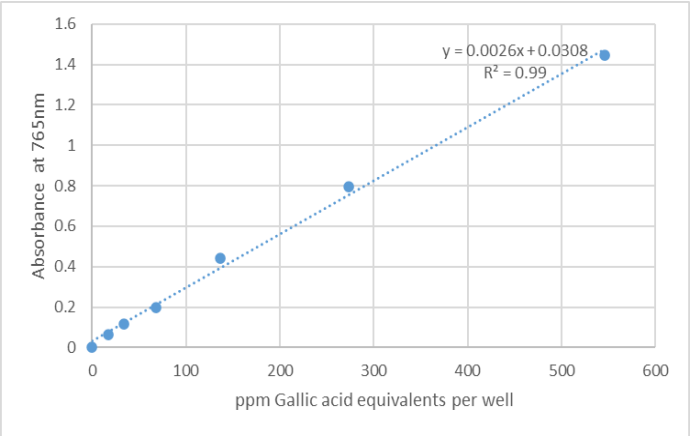


Figure 27. Example of gallic acid standard calibration curve.

3.6.3 Flavonoid Quantification

Extraction of Flavonoids in Pepper

Flavonoid extraction was made from 100 mg powder pepper sample (three biological replicates for each one) weighted in 2 mL microtubes. After the addition of 1.5 mL extraction solvent (80:20 methanol: water v: v with 0.1% butyl-hydroxy-toluene (BHT)), the samples were covered by parafilm and sonicated in ice-water bath with an ultrasonic apparatus for 1 hour at 40°C (Figure 28). The samples were then centrifuged at 10,000 rpm for 5 minutes. Hydrolysis of the bonds between the flavonoids and glycosides was performed by treating 700 µL sample with 350 µL of HCl 3M in screwcap microtubes.

Figure 28. Samples of pepper in the ultrasonic bath under extraction hood.

After vortexing few seconds the samples, they were kept for 1 hour in a thermoblock at 95ºC (Figure 29). The hydrolyzed sample was then centrifuged at 7000 rpm for 5 minutes. Finally, each sample supernatant was filtered through PTFE filters (0.22 µm) membrane directly into a glass vial and analyzed by High Performance Liquid Chromatography (HPLC).

Figure 29. Hydrolysed samples in thermoblock.

HPLC Analysis

Main flavonoids were estimated according to (Bae *et al*., 2012) with some modifications. After acid hydrolysis, the flavonoids were determined in their aglycon form by using HPLC (1220 Infinity LC, Agilent Technologies, Madrid, Spain) coupled to an UV detector (Figure 30). Brisa C18 column (3 µm particle size, 150 × 4.6 mm) (Teknokroma, Barcelona, Spain) was used to separate each flavonoid. The mobile phases consisted of pure water (A) and HPLC-grade methanol (B), (Sigma-Aldrich, St Louis, MO, USA) both with 0.1% formic acid. The flow rate was 0.8 mL/min in a gradient mode. The optimum program elution used was as follow: a linear gradient of 40-10% B (0-10 minutes), 100% B (10-15 min) and a linear gradient of 100-40 % B (15-20 min). The column was equilibrated for 5 min before next injection. The sample injection was 10 µL, the column temperature was set to 30 ºC, and the flavonoids were detected at 360

nm. The separated flavonoids were distinguished by comparing the retention time of individual standards with peaks in sample (Figure 30).

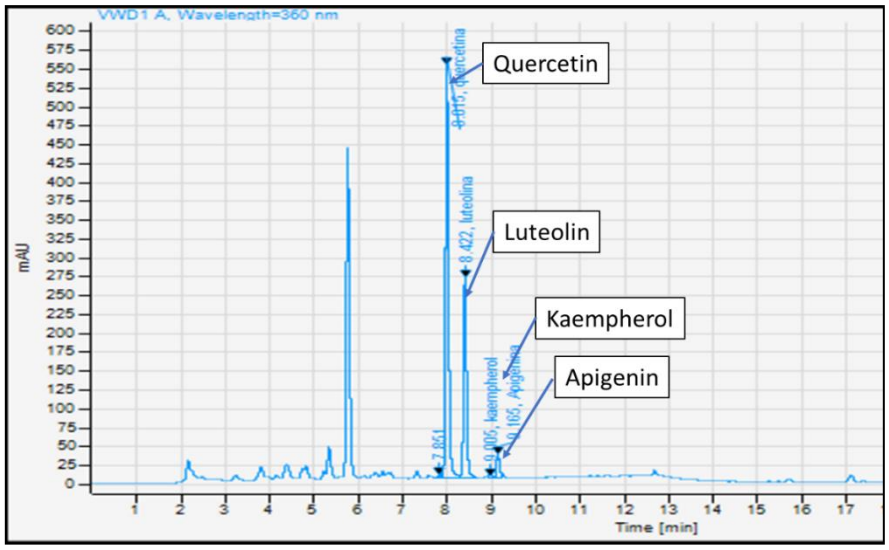


Figure 30. Chromatogram for the detection of the main flavonoids of peppers (quercetin, luteolin, kaempherol and apigenin).

Figure 31. HPLC instrument.

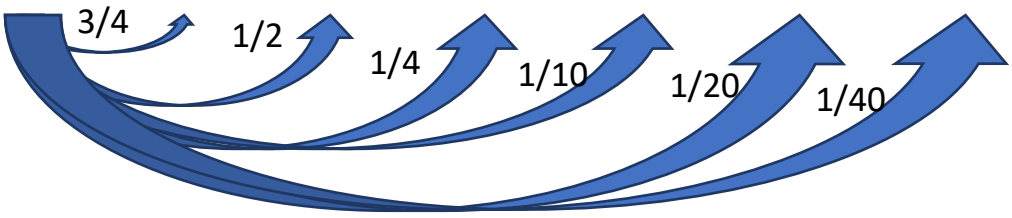
Calibration for Quantification of Flavonoids

The calibration curve was expressed by peak area versus concentration of each flavonoid. To prepare the standard solution, a stock solution containing all the flavonoids was made. The stock solution for quercetin, luteolin, kaempherol and apigenin were prepared from 1045 ppm, 980 ppm 485 ppm and 475 ppm respectively. With the formula C*V=C'*V', (where C and V were initial concentration and volume of solution, C' and V' were final concentration and volume of solution respectively), the quantity of stock solution for CAL1 was calculated with final volume of 5 ml. The dilutions of seven calibration points were then made from CAL 1, the standard was dissolved by methanol as shown in the table below.

Table 4. External standard calibration dilutions for the four flavonoid compounds in ppm.

	CAL1	CAL2	CAL3	CAL4	CAL5	CAL6	CAL7
Q	125.4	94.05	62.7	31.35	12.54	6.27	3.135
L	117.6	88.2	58.8	29.4	11.76	5.88	2.94
K	9.7	7.275	4.85	2.425	0.97	0.485	0.2425
A	47.5	35.625	23.75	11.875	4.75	2.375	1.1875

Dilutions from CAL 1



The regression equation between the known concentration of flavonoids compounds and area units was then calculated (Figure 32).

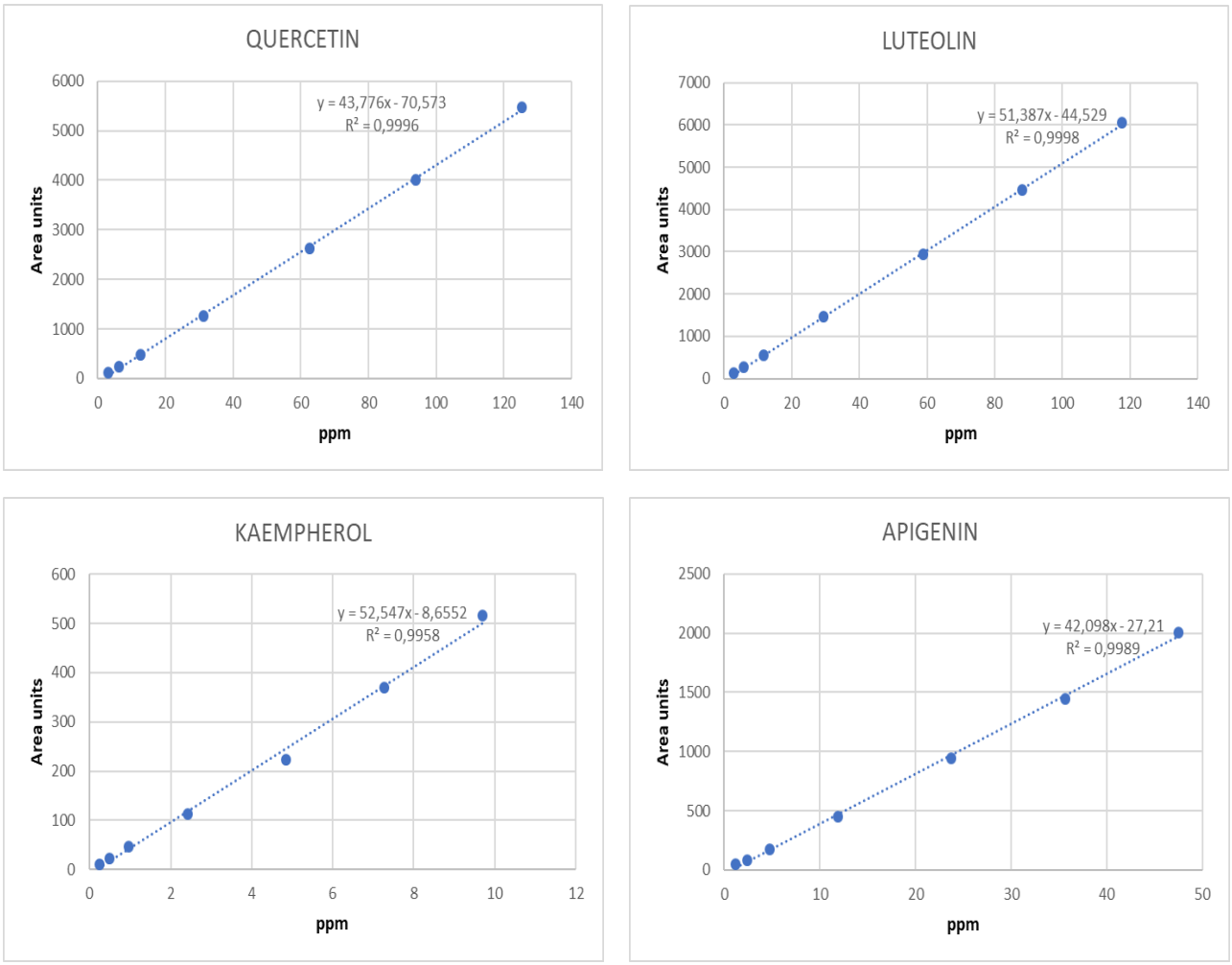


Figure 32. Calibration curves and regression coefficient (R2) for main individual flavonoids.

Each flavonoid value obtained with the calibration curves, was converted to $\mu g/g$ of dry pepper by the equation below, by considering the dilutions applied for hydrolysis (3/2) and extraction solution volume (1.5ml).

$$\frac{\mu g}{g}\ \text{dry pepper} = \text{ppm}\left(\frac{mg}{L}\right) * 1000\frac{\mu g}{mg} * \frac{3}{2}\left(\frac{\text{extraction solution volume (L)}}{\text{dry peppr weight (g)}}\right)$$

3.6.4 Sugars Quantification

Sample Extraction for Sugars

Determination of sugars was based on Rosa-Martínez *et al.*, (2021) with some modifications. Lyophilized sample powder of 100 mg was diluted in 1.5 mL of ultrapure water in a 2 mL microcentrifuge tube. The samples were extracted with a vortex agitator at maximum rpm rotational speed set for few seconds. The samples were put in the ultrasonic bath for 30 minutes at room temperature and centrifugated in 14,000 rpm for 5 minutes. The supernatant was filtered through using 0.22 µm PVDF syringe filters directly to vial and analyzed by HPLC.

HPLC Analysis for Sugars

Simple sugars were analyzed through Agilent 1220-Infinity HPLC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a Refractive Index (RI) detector (Varian Pro Star, model 350, Palo Alto, CA, USA). A RI was used for the detection because simple sugars are not able to absorb or emit light. A stationary phase consisting of a Phenomenex Luna Omega Sugar column, with a pore diameter of 3µm, and dimensions of 150 x 4.6 mm were used. The mobile phase was constituted from mixture of 75:25 v / v acetonitrile: water in an isocratic mode for 15 min. A volume injection of 10 µl, temperature of 35ºC and a flow of 0.8 mL/min were used. The estimated retention time to which fructose migrates was 5.3 min, glucose around 6.1 min. and sucrose, was delayed until approximately 8.2 min (Figure 33).

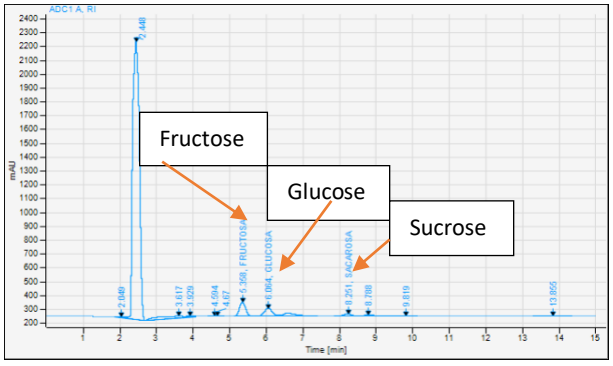


Figure 33. Chromatography of individual sugar components.

Calibration Curves for Sugars

To develop the calibration curves to quantify the concentrations corresponding to the areas of the chromatographic peaks of each sample, a stock solution of fructose, glucose and sucrose with known concentrations was developed. The calibration curve was made from a stock solution of around 20 ppm, 20 ppm and 5 ppm for fructose, glucose, and sucrose respectively. The most concentrated point takes place in 0.5 mL of each stock solution and 0.5mL of pure water (each sugar was diluted ¼ from stock solution). This point was used to make a serial dilution up to 5 times. The equations for each sugar compound were derived from 6 calibration points as shown in Figure 34.

According to these standard curves, the concentration of the three sugars in each sample was estimated from the area obtained. The unit mg/L (ppm) was converted to g/100g of pepper by taking in to account the ¼ dilutions applied to the samples and the water content of the samples through a transformation formula:

$$\text{g/100 g dry of pepper} = \left(\frac{\text{ppm}\left(\frac{g}{L}\right) * volume\ of\ extraction\ solution\ (L)}{Dry\ sample\ weight\ (g)} \right) * 100$$

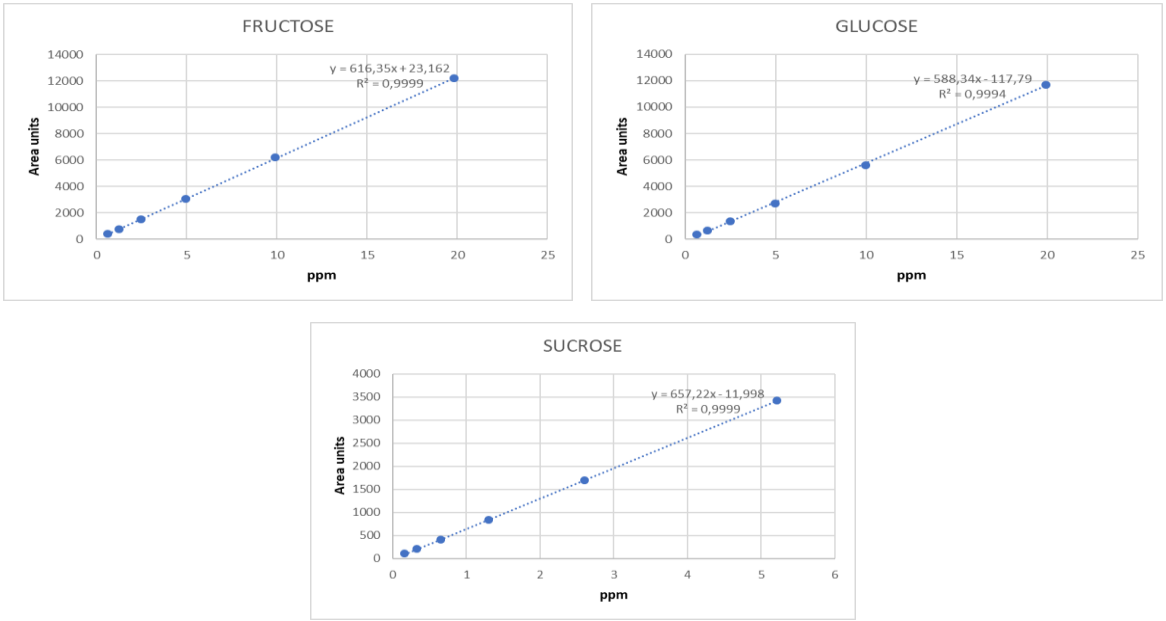


Figure 34. Calibration curves and regression coefficient (R^2) for glucose, fructose, and sucrose.

3.6.5 Ascorbic Acid Analysis

Extraction of Ascorbic Acid

The analysis of ascorbic acid was performed by an HPLC method based on previously validated work of Topuz & Ozdemir (2007) with some modifications. 100 mg of sample powder was extracted with 1.5 mL of 6% metaphosphoric acid cold solution in a 2ml centrifuge tube. The samples were put in an ultrasonic bath for 30 min with water and ice. After that, the samples were centrifugated at 12,000 rpm for 5 min at 5 ºC. The supernatant was filtered using 0.22 µm PVDF syringe filters to HPLC vials for analysis.

Chromatography Conditions

Ascorbic acid was analyzed, in a stationary phase consisting of a C18 Mediterranea Sea column (Teknokroma), with a pore diameter of 3 µm, and dimensions de 150 x 4.6 mm. The mobile phase consisted of 95% phase A (water containing 1% acetic acid) and 5% phase B (methanol) in an isocratic mode for 15 min (Rosa-Martínez *et al*., 2021). A flow of 1mL/min and an injection volume of 5 µL were used. The retention time at which ascorbic acid migrates was around 2.2 min and quantification of ascorbic acid was performed by UV-Vis detector at absorbance wavelength of 254 nm (Figure 35).

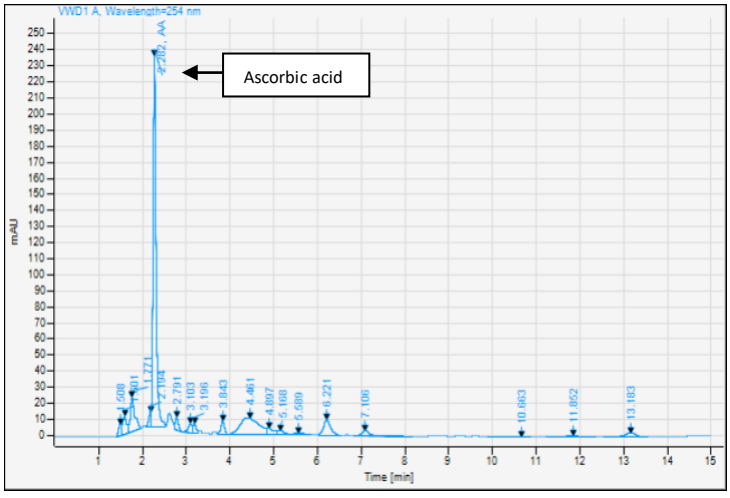


Figure 35. Chromatography output of ascorbic acid.

Calibration and Regression Equation

200 mg of ascorbic acid (purity 99%) was diluted in a cold 6% solution of metaphosphoric acid and made up to 50 mL in a volumetric flask., to make the stock solution 3960 mg/L. To do the calibration curve, 10 calibration points were prepared by making serial dilutions. The estimated retention time was 2.2 min. The calibration curve obtained is shown below (Figure 36).

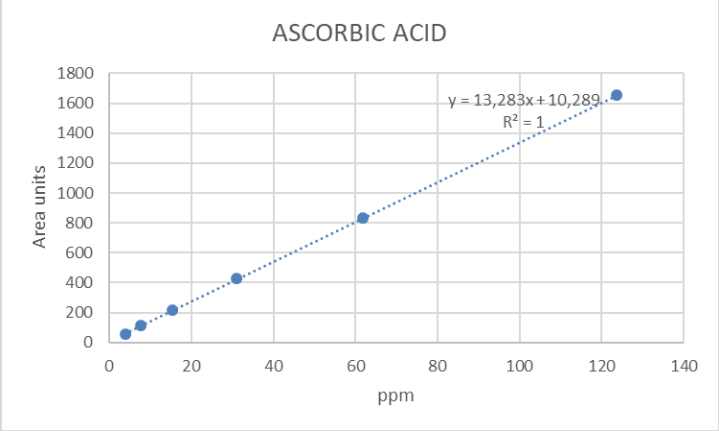


Figure 36. Calibration curve and regression coefficient (R2) for ascorbic acid.

3.7 Statistical Analysis

The statistical program Stat graphics Centurion 18 (Stat graphics Technologies, The Plains, VA, USA) were used to analyze phenotypic and analytical laboratory collected data. The mean and standard error of means were calculated. The main factors of the experiment variety and maturity as well as their combined interaction were evaluated by analysis of variance (ANOVA). General ANOVA was performed for effects of variety and ripening stage. In addition, the ANOVA was also distinguished between unripe and fully ripe stages. Student–Newman–Keuls (SNK) test was used to determine significance differences between means at $p < 0.05$. Pearson's correlation test was also applied to detect significant bivariate relations between flavonoids and sugars for the green ripe and fully ripe stages.

4. RESULTS AND DISCUSSION

4.1 Phenotypic Characterization

4.1.1 Qualitative Traits

Descriptive Study of Qualitative Morphological Fruit Traits

In the phenotypic characterization, high variations were observed among varieties for different pepper fruit physical traits. The main qualitative traits responsible for variation were fruit color at intermediate and mature stage, fruit shape, fruit shape at pedicel attachment and at blossom end, and fruit cross sectional corrugation.

Table 5. Qualitative fruit morphological traits of green and fully ripe stages (IPGRI, 1995).

Effects	FCI	FCM	FSH	FSP	FSB	FSU	FCC
Green ripe							
Variety							
Bola	Green	Dark red	Almost round	Truncate	Blunt	Smooth	Slightly corrugated
Bierzo	Green	Red	Campanulate	Lobate	Sunken	Smooth	Slightly corrugated
Guindilla	Green	Red	Elongate	Obtuse	Pointed	Smooth	Slightly corrugated
Largo de Reus	Green	Red	Blocky	Lobate	Sunken	Smooth	Intermediate
Blanco de Villena	White	Red	Triangular	Lobate	Blunt	Smooth	Slightly corrugated
Fully ripe							
Bola	Green	Dark red	Almost round	Truncate	Blunt	Smooth	Slightly corrugated
Bierzo	Green	Red	Campanulate	Lobate	Sunken	Smooth	Slightly corrugated
Guindilla	Green	Red	Elongate	Obtuse	Pointed	Smooth	Slightly corrugated
Largo de Reus	Green	Red	Blocky	Lobate	Sunken	Smooth	Intermediate
Blanco de Villena	White	Red	Triangular	Cordate	Blunt	Smooth	Intermediate

FCI= Fruit color at intermediate stage FCM=Fruit color at mature stage, FSH=Fruit shape, FSP=Fruit shape at pedicle attachment, FSB=Fruit shape at blossom end, FSU=Fruit Surface, FCC=Fruit cross sectional corrugation

Fruit color at intermediate stage showed a green color for all varieties except for Blanco de Villena which was white color. Fruit color was changed to red for all varieties, and dark red color was observed for variety Bola during fruit color at mature stage. There was quite difference in fruit shape, fruit shape at pedicel attachment and at blossom end among the tested varieties (Table 5). Regarding fruit cross sectional corrugation, variety Bierzo, Bola and Guindilla showed

slightly corrugated, Blanco de Villena and Largo de Reus were intermediate. While in terms of fruit surface, all the varieties showed smooth surface.

4.1.2 Quantitative Traits

Effects of the Variety, Ripening Stage, and their Interactions on Quantitative Fruit Morphological Traits

The analysis of fruit quantitative traits showed that higher variation was contributed by variety effect and in some extent by the effect of maturity based on the value of their mean square (Table 6). There was a highly significant effect of variety for all the tested quantitative traits: fruit length, diameter, weight, number of locules, pedicel length, fruit fresh and dry weight and total yield. A highly significant effect of ripening stage was also observed for fruit length, fruit diameter and fruit weight. To distinguish effect of variety in each maturity group, separate ANOVA also made for the green ripe and fully ripe quantitative traits as shown in Table 6.

The specific analysis of variance for quantitative fruit traits showed highly significant effect of variety at both ripening stages for fruit length, fruit diameter, fruit weight, number of locules and pedicel length and total yield. While there had no significant effect of variety for fruit dry weight at the green ripe stage and fruit fresh weight at the fully ripe stage (Table 6).

Table 6. General and Specific ANOVA for quantitative fruit characters of main and interaction effects.

Main effects		**FL**	**FD**	**FW**	**NL**	**PL**		**FFW**		**FDW**		**YLD**
	DF	MS	MS	MS	MS	MS	DF	MS	DF	MS	DF	MS
Variety(V)	4	797***	187***	81029***	16***	14***	4	65151**	4	3507*	4	4889410**
Maturity(M)	1	74***	35***	38243***	3***	0.8NS	1	50753NS	1	23NS	1	1543600NS
Interaction												
V x M	4	6.8NS	5.0***	6909***	2.3***	3.1***	4	66575**	4	471*	4	2874350*
Residual	216	4.14	0.70	602	0.4	0.5NS	38	16464	38	124	20	1000860
Green_ripe												
Main effects												
Variety(V)	4	429***	126***	39099***	8.2***	16.2***	4	90343**	4	193NS	4	5512260*
Residual	137	5.2	1.0	828	0.4	0.5	23	16747	145	128	10	953020
Fully_ripe												
Main effects												
Variety(V)	4	435***	97***	49935***	8.9***	8.9***	4	47282NS	4	567*	4	2251490NS
Residual	79	2.29	0.2	210	0.3	0.4	15	16029	15	119	10	1048710

*FL=Fruit length (cm), FD=Fruit diameter (cm), FW=Fruit weight (g), PL=Pedicele length (cm), NL=Number of locales, FFW=Fruit fresh weight (g), FDW=Fruit dry weight (35º C - 40º C) and YLD=Yield (kg/ha) and FWP=Fruit weight per plant (g). DF, degrees of freedom; MS, mean square. NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

Variations in Quantitative Fruit Morphological Traits among Varieties

Among the tested varieties, variety Largo de Reus showed higher values for fruit length, fruit diameter and fruit weight at both ripening stages. Guindilla was the second variety with a higher fruit length, although the difference was not statistically significant compared to Largo de Reus. Interms of fruit diameter and fruit weight, Bierzo and Blanco de Villena respectively showed higher values following Largo de Reus.

The analysis for green ripe showed that variety Largo de Reus had higher values for fruit length (130 mm), followed by Gundilla (127 mm) and Blanco de Villena (98 mm). Higher value for fruit diameter and fruit weight were also observed in variety Largo de Reus (65 mm and 89 g respectively), followed by Bierzo (60 mm and 74 g respectively). Meanwhile, Guindilla had the least fruit diameter (13 mm) and fruit weight of 9.5 g. Concerning pedicel length, the higher value was recorded from Largo de Reus (41 mm) and Bierzo (39 mm). Blanco de Villena was the second variety for higher pedicel length (3.3 cm), while Bola (2.6 cm) and Guindilla (2.4 cm) had the lowest pedicel lengths.

Moreover, a higher number of locules were recorded from variety Blanco de Villena (3.5), Bola (3.4) and Largo de Reus (3.3), without statistical differences among them. Variety Bierzo (2.9) ranked second for number of locules, followed by the least (2.2), which was observed in variety Guindilla. Regarding fruit fresh weight, the range spanned almost 100 g for Guindilla and 400 g for Blanco de Villena. No statistical differences were observed among Balnco de Villena (396 g), Bierzo (349 g), and Largo de Reus (316 g) among the tested varieties (Table 7).

Moving on to the fully ripe fruits, most of the characters related to fruit size and dimension parameters exhibited an increase with maturity. However, both fruit fresh weight and fruit dry weight showed a decrease with maturity. This suggests that metabolic activities such as water loss through transpiration, respiration involving the breakdown of organic compounds, and conversion of starches to sugars can contribute a decrease in fruit weight (Lufu et al., 2019).

Variety Bola and Guindilla, on the other hand were the exceptional varieties they showed an increase in fruit fresh and dry weight at fruit ripe stage (Table 7). This may happen due to genetic variations linked to skin permeability allowing water to enter the fruit. This factor could contribute to the observed increase in fruit weight.

Similar to the green ripe stage, in fully ripe fruits, variety Largo de Reus showed the highest values for fruit length (149 mm), fruit diameter (87 mm) and fruit weight (177 g), followed by Guindilla (141 mm) for fruit length and Bierzo (82 mm and 135 g) for fruit diameter and fruit weight respectively. As usual, variety Bola had the smallest value for fruit length (41mm) and fruit weight (23 g), with no statistical difference compared to Guindilla (9.5 g). Guindilla (13 mm) exhibited the smallest fruit diameter among the tested varieties.

Regarding the number of locules, variety Largo de Reus had the highest value (4.7), while the lowest value (2.1) was observed in variety Guindilla, as usual in the green ripe fruits. The highest pedicel length (42 mm) was observed for Blanco de Villena among the tested varieties. In contrary to fruit fresh weight, a significance difference was observed for fruit dry weight. Variety Bola showed the highest value (35 g), followed by Blanco de Villena and Guindilla (17.5 g and 17.0 g) respectively, with no statistical difference among them (Table 7).

Interms of total yield, encompassing both green ripe and fully ripe fruits, a significant difference was observed among the varieties. The highest total yield was recorded from varieties Largo de Reus and Blanco de Villena, yielding 5900 kg/ha and 5500 kg/ha respectively. Following varieties were Bola (4300 kg/ha) and Bierzo (3800 kg/ha) with no significant differences among them. In comparison, variety Guindilla had the lowest total yield of 1800 kg/ha among the tested varieties.

A decline in yield components, such as fruit fresh weight and fruit dry weight was observed in most of the varieties during the fully ripe stage in comparison to the green ripe fruits. This phenomenon is consistent with findings from previous studies (Wiedenfeld, 2019). Where an increase in maturity leads to a decrease in fruit weight due to metabolic activities within the plant, such as transpiration and respiration.

Table 7. Quantitative fruit morphological traits of green ripe and fully ripe stage, average value, and standard error.

Effects	FL	FD	FW	PL	NL	FFW	FDW	TYLD [1]
Green_ripe								
Variety								
Bola	3.5±0.42c	3.5±0.18c	14.7±5.25b	2.6±0.13c	3.4±0.12a	186.3±52.83ab	15.8±4.61a	
Bierzo	8.9±0.42b	5.9±0.18a	73.8±5.25a	3.9±0.13a	2.9±0.12b	349.4±57.87a	20.0±5.05a	
Guindilla	12.7±0.42a	1.3±0.18d	9.5±5.25b	2.4±0.13c	2.2±0.12c	95.7±52.83b	9.7±4.61a	
Largo de Reus	13.0±0.45a	6.5±0.19a	89.5±5.64a	4.1±0.14a	3.3±0.13a	316.2±57.87a	18.2±5.05a	
Blanco de Villena	9.8±0.45b	4.9±0.19b	71.8±5.64a	3.3±0.14b	3.5±0.13a	396.3±52.83a	25.2±4.61a	
Significance	***	***	***	***	***	**	NS	
Fully_ripe								
Bola	4.1±0.27c	4.0±0.08d	22.7±2.6d	2.6±0.11b	3.4±0.091b	347.2±51.69a	35.2±4.45a	4300ab
Bierzo	11.0±0.87b	8.2±0.24b	134.7±8.3b	3.4±0.34b	3.7±0.3b	121.5±89.52a	6.0±7.72b	3800ab
Guindilla	14.1±0.27a	1.3±0.08e	9.5±2.6d	2.6±0.11b	2.1±0.09c	157.0±128.5a	17.0±4.45ab	1800b
Largo de Reus	14.9±0.87a	8.7±0.24a	177.0±8.4a	2.8±0.0.34b	4.7±03a	85.0±89.52a	5.5±7.72b	5900a
Blanco de Villena	12.4±0.35b	5.8±0.09c	107.7±3.4c	4.2±0.14a	3.0±0.12b	278.3±63.3a	17.5±5.45ab	5500a
Significance	***	***	***	***	***	NS	*	*

*FL=Fruit length (cm), FD=Fruit diameter (cm), FW=Fruit weight(g), PD=Pedicele length(cm), NL=Number of locales, FFW=Fruit fresh weight (g), FDW=Fruit dry weight (35º C - 40º C) and YLD= Yield(kg/ha), FWP= Fruit weight per plant (g). DF, degrees of freedom; MS, mean square. NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

1 Total yield was measured as the sum of all the green ripe and fully ripe peppers harvested, although displayed in the fully ripe rows.

4.2 Bioactive Compound and Nutritional Quality Analysis

4.2.1 Carotenoids

Analysis of Carotenoids

The analysis for carotenoid was conducted solely on fully ripe fruits, as the carotenoid content in green ripe fruits is nearly negligible. Highly significant differences were observed among the tested varieties in terms of red carotenoid, yellow carotenoid, and total carotenoid (Table 8). Among these varieties, Bola exhibited the highest content of red, yellow, and total carotenoids (2029, 1093 and 3122 µg/g DW, respectively). In this study, the content of red carotenoid ranged between 690 µg/g DW and 2029 µg/g DW for Largo de Reus and Bola, respectively. Bierzo and Guindilla showed the highest red carotenoids levels (1914 and 1885 µg/g DW, respectively), following Bola with no significance difference among them (Table 9).

Table 8. ANOVA for red carotenoid, yellow carotenoid, and total carotenoid (µg/g).

Source of variations	**Red Carotenoid**		**Yellow Carotenoid**		**Total Carotenoid**	
	DF	MS	DF	MS	DF	MS
Variety	4	4948010***	4	1826310***	4	1225490***
Error	64	100175	64	25669.2	64	204467

*Df, degrees of freedom; MS, mean square. NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

This high carotenoid content of Bola, coupled with lower flavonoid content, coincides with studies suggesting that high content of carotenoid may be observed when there is less lipid and flavonoid syntheses (Feng et al., 2022). The findings of this study are consistent with and indicate higher carotenoid content compared to the results reported by Topuz & Ozdemir (2007). Their study reported red, yellow, and total carotenoid contents of 1340, 1050 and 2390 mg/kg, respectively, for dry matter of some selected pepper cultivars.

In previous studies, variety Bola showed total carotenoids content of 5018 mg/kg in dry weight basis (Mínguez-Mosquera & Hornero-Méndez, 1994), which is slightly higher compared to our

findings. Additionally, when compared to red pepper cultivars with carotenoid content reaching 8797 mg/kg DW (Hornero-Méndez et al., 2000), the red carotenoid content in the present study is significantly lower. Conversely, the total carotenoid content obtained in this experiment is significantly higher than the carotenoid content of selected sweet bell pepper fruits, which resulted 157 mg/kg DW under organic farming conditions (Hallmann & Rembial Kowska, 2012). The variations among the reported carotenoid contents of these peppers may be due to their differences in genotype, maturity stage and drying process.

Regarding yellow carotenoid, a highly significant variation was observed, ranging from 330 µg/g-1095 µg/g DW for Largo de Reus and Bola, respectively. Variety Guindilla also had higher yellow carotenoid content (992 µg/g DW), ranking second to Bola, and no statistical significance difference was observed between them (Table 9). The lowest red, yellow and total carotenoid content was observed in Largo de Reus (690, 330 and 1020 µg/g DW, respectively), followed by Blanco de Villena (962, 393 and 1355 µg/g DW, respectively).

Table 9. Red carotenoid, yellow carotenoid, and total carotenoid (µg/g DW), average content and standard error for ripe fruits.

Variety	Red carotenoid	Yellow carotenoid	Total carotenoid
Bola	2029 ±74.6a	1095 ±37.76a	3122±106.58a
Bierzo	1914 ±129.21a	538±65.41b	2452±184.6b
Guindilla	1885±74.6a	992±37.6a	2877±106.58a
Largo de Reus	690 ±110.5c	330±53.4bc	1020±1150.73c
Blanco de Villena	962 ±74.6b	393±37.76c	1355±106.58c
Significance	***	***	***

*NS, *, ** and *** indicate not significant for a probability of $P > 0.05$ and significant for $P < 0.05$, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of $p \leq 0.05$).*

4.2.2 Flavonoids

Analysis of Variance for Flavonoid Contents and Total Phenolics

The general ANOVA revealed that the variation for individual and total flavonoid content, as well as for total phenolics, was mainly due to variety according to their percent value of mean square. The maturity stage also contributed to this variation to some extent, particularly for kaempferol, apigenin, luteolin and total phenolics. An overall significant interaction effect was observed for both individual and total flavonoids, as well as for total phenolics (Table 10).

According to the specific ANOVA for each maturity group, a significant effect of variety was observed for each individual and total flavonoid in both green ripe and fully ripe fruits. Interms of total phenolics, the variety exhibited a significant effect at the green ripe stage, in contrast to the fully ripe fruits (Table 10).

Flavonoids in Green Ripe and Fully Ripe Fruits

Flavonoid aglycons were quantified in green ripe and fully ripe pepper varieties for quercetin, kaempferol, apigenin and luteolin. The highest flavonoid content was contributed by quercetin 67.5% and luteolin 26.9%, in green ripe fruits. While apigenin and kaempferol contributed considerably lower amount for total flavonoids (Table 11).

The analysis of main flavonoids at green ripe fruits showed a significant difference among varieties for both individual flavonoid components and total flavonoid content (Table 11). Variety Guindilla showed the highest concentrations of total flavonoids, which was 1856 µg/g DW, while significantly lower content of 543 µg/g DW and 554 µg/g DW were observed in varieties Bola and Blanco de Villena, respectively. Furthermore, a considerable variation was observed in the concentration of each individual flavonoid component among the tested varieties. The concentration of quercetin ranges from 288 and 1233 µg/g DW for varieties Bola and Guindilla, respectively, and luteolin ranged from 163 to 528 µg/g DW for varieties Largo de Reus and Guindilla, respectively. The content of apigenin ranged between 35 and 84 µg/g DW, and kaempferol between 5 and 11 µg/g DW, both for Blanco de Villena and Guindilla, respectively (Table 11).

Table 10. General and specific ANOVA for individual and total flavonoids (µg/g DW) and total phenolics (mg eq gallic/100g).

Main effects		**Quercetin**	**Kaempferol**	**Apigenin**	**Luteolin**	**Total flavonoid**	**Total phenolics**
	Df	*MS*	*MS*	*MS*	*MS*	*MS*	*MS*
Variety (V)	4	3925660***	225***	14016***	858352***	8323440***	1226950***
Maturity (M)	1	72520^{NS}	104***	1097*	137169***	20850^{NS}	20225000***
Interaction							
V x M	4	886751***	26.2***	779**	53258.6***	1155210***	701169**
Error	140	67210	3.7	17	5568.44	1065119	190895
Green_ripe							
Main effects							
Variety	4	2986530***	103.9***	6708.52***	377289***	5572960***	1865450***
Error	76	69810.3	1.7	146.31	2703.1	90953.9	295098
Fully_ripe							
Main effects							
Variety	4	1819440***	139.3***	8090.89***	539633***	3903850***	131213^{NS}
Error	64	64122	6.03	209.49	8971.03	125003	67154.1

*Df, degrees of freedom; MS, mean square. NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

Compared to previous work of Bae et al. (2012) on the extraction efficiency and validation of flavonoids, quercetin and luteolin content of 357 µg/g DW and 59 µg/g DW, respectively, in paprika varieties, the results of the current study are notably higher. Additionally, in another study by Miean & Mohamed (2001), quercetin and apigenin content of 448 µg/g and 272 µg/g were observed in bell peppers, but no luteolin was detected. Moreover, in recent work by Ribes-Moya et al. (2020), similar differences among flavonoid components in green ripe *Capsicum* fruits were reported, with quercetin and luteolin ranging from 2 to 72 mg/kg FW and 29 to 220 mg/kg FW, respectively. However, in our study, a high dominance of the mean quercetin content was found, which contrasts with their findings. In addition, the average value of each main flavonoid in this experiment was higher than previously reported studies.

Table 11. Individual and total flavonoids (µg/g DW) and total phenolics (mg eq gallic/100g DW) average content and standard error at green ripe and fully ripe stage.

Effects	Quercetin	Kaempferol	Apigenin	Luteolin	Total flavonoid	Total phenolics
Green_ripe						
Variety						
Bola	288±62d	5.3 ± 0.3c	44.6 ±2.8b	204±12.3c	543±71d	2457±128a
Bierzo	978±68b	8.2±0.3b	45.1 ±3.1b	291±13.4b	1322±77b	1773±140b
Guindilla	1233±62a	10.9 ±0.3a	84.1±2.9a	528±12.3a	1856 ±71a	1631±128b
Largo de Reus	641±76c	8.9 ±0.4b	40 ±3.5b	163±15.0c	853 ±87c	1863±157b
Blanco de Villena	320±62d	5.2 ±0.3c	35.4± 2.9b	193±12.3c	554±71d	2124±128ab
Significance	*******	*******	***	***	***	***
Fully_ripe						
Bola	237±57c	6.3 ±0.6c	47±3.4c	239 ±22.3c	529±83c	1230±61a
Bierzo	808±103b	9.4 ±1.0b	45±5.9c	308 ±38.7bc	1171±144b	1081±106a
Guindilla	727±60b	10.7±0.6b	88±3.4a	618±22a	1444±83ab	1081±61a
Largo de Reus	1161±84a	14.4±0. 8a	66±56.4b	375±31.6b	1616±118a	1345±86a
Blanco de Villena	292±60c	6.5±0.6c	32±3.4d	163±22.3d	493 ±83c	1188±61a
Significance	***	*******	***	***	***	NS

*NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

At the green ripe stage, Guindilla had the highest quercetin content (1233 µg/g DW), followed by Bierzo (978 µg/g DW). The lowest quercetin concentrations were observed in Bola (288 µg/g DW) and Blanco de Villena (320 µg/g DW) with no statistical difference between them. Next to Guindilla, Largo de Reus (8.9 µg/g DW) and Bierzo (8.2 µg/g DW) exhibited the second highest kaempferol content. On the other hand, Blanco de Villena and Bola had the lowest content, which were 5.2 and 5.3 µg/g DW, respectively. Apigenin and luteolin contents were higher in variety Guindilla (84 µg/g DW and 528 µg/g DW, respectively), followed by Bierzo, with contents of 45.1 µg/g DW and 291 µg/g DW, respectively. Meanwhile, variety Largo de Reus showed lowest content for apigenin and luteolin (40 µg/g DW and 163 µg/g DW), respectively.

A previous study by Ribes-Moya et al. (2020) reported total flavonoid content ranged from 38 – 287.1 mg/kg FW at the green ripe stage in pepper collections, including Bola and Guindilla

varieties, under different growing systems and ripening stage conditions. Nonetheless, our results were much higher than the reports mentioned, indicating that organic farming jointly with agroforestry condition had significant impact for plant secondary metabolites production. This is supported by evidence from studies focused on the effect of light intensity on polyphenolic composition under Mediterranean agroforestry conditions. An appropriate light control within agroforestry systems may enhance the secondary metabolite content of plants (Re et al., 2019).

Regarding flavonoids in fully ripe fruits, there was a decrease in individual and total flavonoid content for all the varieties, except for Largo de Reus (Table 11). In contrast, variety Largo de Reus exhibited an increase for flavonoid content, approximately by 2-fold generally for total flavonoids. The result of decreasing in flavonoid content with maturity contradicts some previous works that suggest flavonoid content increases with maturity (Lee et al., 1995). However, certain studies have reported that green ripe fruits may show higher values than fully ripe fruits (Howard et al., 2000). Studies of different pepper cultivars harvested at different times have also shown a decrease on flavonoid and phenolics content in fully ripe fruits compared to less ripe/mature fruits (Ghasemnezhad et al., 2011). Flavonoid losses during maturation could be attributed to metabolic conversion to secondary phenolic compounds (Hoesel & Studies, 1979) or degradation through enzymatic action (Jiménez et al., 1998).

In the fully ripe fruits, the higher percentage of flavonoid were contributed by quercetin and luteolin (61.3% and 32.4%, respectively), as usual in the green ripe fruits. On the reverse, apigenin and kaempferol were present in lower contents. Highly significant difference was observed among varieties on each individual flavonoid components and total flavonoid. The content of quercetin was ranged from 237 and 1161 µg/g DW for Bola and Largo de Reus, respectively. This amount is higher compared to previous studies, which reported a range of 23 to 398 mg/kg dry matter for fully ripe fruits under organic farming conditions (Ribes-Moya et al., 2018).

The content of Luteolin was ranging between 163 µg/g DW and 618 µg/g DW and apigenin from 32 µg/g DW and 88 µg/g DW for Blanco de Villena and Guindilla, respectively (Table 11). The content of luteolin here under organic agroforestry condition is higher compared to the one reported in previous work of Hallmann et al. (2012), 84 mg/kg under organic growing condition. Regarding kaempferol, higher content was observed from variety Largo de Reus (14.4 µg/g

DW), while Bola and Blanco de Villena had lowest contents of 6.3 µg/g DW and 6.5 µg/g DW, respectively. Similar studies under organic farming condition including the varieties Bola and Bierzo reported, luteolin content ranging from 212 to 639 µg/g DW, while the content of luteolin for Bierzo variety (638.6 µg/g DW) at fully mature stage dry weight condition in this study (Ribes-Moya et al., 2018) significantly higher than the current study. However, the quercetin content of Bierzo in the current study (808 µg/g DW) is much higher than the previously reported, which was 398 µg/g DW at the fully ripe stage. This may be due to different agronomic factors, such as drying process and growing conditions, as if the current study was conducted under organic agroforestry condition.

In terms of total flavonoids, variety Largo de Reus had the highest values (1616 µg/g DW), followed by Guindilla (1444 µg/g DW) and Bierzo (1171 µg/g DW), at the fully ripe stage. This value is higher compared to the previously reported total flavonoid content of 708 µg/g DW in fully ripe fruits of selected bell pepper varieties under organic conditions (Hallmann & Rembial kowska, 2012). Even if, a higher content of total flavonoids ranging from 6541 to 23110 mg CE/kg DW were reported (Sricharoen et al., 2017).

Significant differences were also observed among varieties for individual flavonoid compounds. The highest quercetin value was found in Largo de Reus (1161 µg/g DW), followed by Bierzo (808 µg/g DW) and Guindilla (727 µg/g DW). Guindilla had the highest content of luteolin (618 µg/g DW), followed by Largo de Reus and Bierzo (375 and 308 µg/g DW, respectively). Variety Guindilla also had highest content of apigenin (88 µg/g DW), followed by Largo de Reus (66 µg/g DW). The highest kaempferol content was observed in Largo de Reus (14.4 µg/g DW), followed by Guindilla with 10.7 µg/g DW.

Total Phenolics in Green Ripe and Fully Ripe Fruits

Organic pepper fruits are characterized by a significantly higher content of phenolics and flavonoids, as evidenced by previous works (Hallmann & Rembial kowska, 2012), as well as by the current study (Table 11). Considerable variation was found among genotypes regarding total phenolics on green ripe and fully ripe maturity groups. A significantly higher content of total phenolics was recorded, which was 2457 mg GAE/100g DW compared to certain previous

works. Total phenolics of 954 mg/kg DW reported in selected bell pepper fruits under organic farming conditions (Hallmann & Rembial kowska, 2012), and 43 µg/g dry weight total phenolics in profile of colored sweet peppers (Thuphairo et al., 2019) .

Regarding the analysis of green ripe fruits, the total phenolic content ranged from 1631 and 2457 mg GAE/100g DW for Guindilla and Bola, respectively. Variety Blanco de Villena showed a higher total phenolic content (2124 mg/100g DW), next to Bola, with no significant difference between them. Similarly, significantly higher content of phenolic was reported in studies focusing on intra and inter species variations influence on three fruit segments of chili pepper (Zamljen et al., 2021). In the study, the total phenolic content ranged from 2599 mg/100 DW in 'Chili AS- Rot' to 7767 mg/100 g DW in 'Carolina Reaper'. Higher total phenolics were also reported ranging from 2096 to 7689 µg/g at dry weight condition, in the green and red mature fruits, respectively (Hamed et al., 2019). The species *C. annuuum*, specifically cultivar cayenne, exhibited the highest total phenolic content (2554 mg/100g DW) in the pericarp, surpassing all other cultivars. The result is consistence with our findings, which were obtained under organic farming conditions.

In the analysis for fully ripe fruits, total phenolics content was dependent on the maturity stage. Green ripe fruits had more phenolic content than fully ripe fruits; as the fruits matured, their phenolic content decreased. This is in agreement with studies on phenolic compounds of different color bell peppers, where green peppers contained higher levels of luteolin conjugates than red and yellow peppers (Zhang & Hamauzu, 2003), and in the study focused four colored sweet peppers higher phenolics were recorded from green fruits compared to the red, orange and yellow fruits (Thuphairo et al., 2019). Additionally, studies focused on different pepper cultivars in different harvest times have shown that high phenolic content is observed during the early ripe stage (Ghasemnezhad et al., 2011). However, this finding contradicts previous research on the antioxidant activity of main phenolic compounds isolated from hot peppers (Materska & Perucka, 2005) and (Lee et al., 1995).

There was no significant effect variety on total phenolic content at fully ripe stage. While significantly higher values were recorded, ranging from 1081 and 1345 mg/100 g DW for Guindilla and Largo de Reus, respectively. This higher content of phenolics significantly compare with studies reported on species of *capsicum chinese* with an average content of three

fruit parts at 1350 mg GAE/100 g DW, and in a cross between *capsicum chinense* and *frutenscens* at 1396 mg GAE/100 g DW for total phenolics (Zamljen et al., 2021). Much higher total phenolic content, ranging from 6,392 to 12,115 mg CE/kg DW, has also been reported for different varieties of hot chili peppers (Sricharoen et al., 2017).

4.2.3 Ascorbic Acid and Sugars

Analysis of Variance for Ascorbic Acid and Sugars

The ascorbic acid content was analyzed in both green ripe and fully ripe pepper fruits at the dry weight conditions. The general ANOVA indicated that there was no significant effect of variety and maturity for ascorbic acid, but an interaction effect was observed between the two factors. In the specific ANOVA for green ripe stage, a significant effect of variety on ascorbic acid was found, while no significant difference was observed in the fully ripe fruits (Table 12).

Regarding individual sugar and total sugars, effects of variety were observed on glucose and sucrose content, and effects of maturity were observed on fructose, glucose, and total sugars.

According to the total mean squares percentage, the variation in glucose, fructose and total sugars was mainly due to the ripening stage, with variety contributing to a lesser extent, particularly for glucose and sucrose. There was no overall interaction effect observed between variety and ripening stage for each individual sugar and total sugar content, except for sucrose.

Furthermore, to gain a deeper understanding of the ripening stages effect within each variety, further ANOVA analyses were performed, by considering the two maturity groups: green ripe and fully ripe stages. The specific ANOVA showed a highly significant variety effect on sucrose content during the green ripe stage, while no significant effect was observed on the remaining individual and total sugar contents. In contrast, during the fully ripe stage, a variety effect was observed only for individual sugar glucose content, differing from the results observed during the green ripe stage.

Table 12. General and specific ANOVA corresponding to each ripening stage for ascorbic acid, fructose, glucose, sucrose, and total sugars.

Main effects		**Ascorbic acid**	**Fructose**	**Glucose**	**Sucrose**	**Total sugars**
	Df	MS	MS	MS	MS	MS
Variety(G)	4	2227 ^{NS}	25^{NS}	22**	1.2**	64 ^{NS}
Maturity (M)	1	1243 ^{NS}	103**	79***	0.02 ^{NS}	368**
Interaction						
G x M	4	12269***	27 ^{NS}	11.00^{NS}	1.35***	79 ^{NS}
Error	70	1538	13	5.11	0.25	32
Green_ripe						
Main effect						
Variety	4	12210.1***	12^{NS}	8.04^{NS}	2.14***	30^{NS}
Error	49	1360	5.5	3.48	0.28	18
Fully_ripe						
Main effect						
Variety	4	2647^{NS}	34.97^{NS}	23.79*	0.43^{NS}	106.65^{NS}
Error	41	1751	21	7	0.21	49

*Df, degrees of freedom; MS, mean square. NS, *, ** and *** indicate not significant for a probability of P > 0.05 and significant for P < 0.05, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of p ≤ 0.05).*

Ascorbic Acid in Green Ripe and Fully Ripe Fruits

At the green ripe stage, there was a highly significant difference in ascorbic acid content among varieties, in contrast to the fully ripe stage (Table 13). Ascorbic acid content ranged from 15 to 93 mg/100g DW for ‘Largo de Reus’ and ‘Guindilla’, respectively. Variety ‘Bola’ was the second-highest content of 61 mg/100 g DW following ‘Guindilla’, with no significance difference between them. In previous study, including the varieties Bierzo, Bola and Guindilla under different growing environments, variety Bola and Guindilla showed an increment with maturity from 55.2 to 129.9 mg/100g Fw and 29.8 to 123.2 mg/100g FW, respectively under

organic growing conditions (Ribes-Moya et al., 2018). While in the current study the reverse was observed for both varieties decreasing in ascorbic acid content with maturity.

Table 13. Ascorbic acid (mg/100 g, DW), individual and total sugar content, average content, and standard error (g/100g, DW) at unripe and ripe stage.

Effects	Ascorbic acid	Fructose	Glucose	Sucrose	Total Sugars
Green_ripe					
Variety					
Bola	61 ±11b	5.4± 0.7a	8.8± 0.5a	0.014 ± 0.2b	14.1± 1.2a
Bierzo	24 ± 12c	3.9± 0.7a	7.3± 0.6a	0.04 ± 0.2b	11.3 ± 1.5a
Guindilla	93 ± 11a	6.2± 0.7a	7.2± 0.5a	1.01± 0.2a	14.4 ± 1.2a
Largo de Reus	15 ± 13c	3.7± 0.8a	7.4± 0.7a	0.1 ± 0.2b	11.3± 1.6a
Blanco de Villena	21± 11c	5.7± 0.7a	8.9 ± 0.5a	0.09 ± 0.2b	14.7 ± 1.2a
Significance	***	NS	NS	***	NS
Fully_ripe					
Variety					
Bola	42± 12a	8.1± 1.33a	9.7± 0.8ab	0 ± 0.1a	17.9±2.02a
Bierzo	77±21a	3.6± 2.31a	9.1± 1.3ab	0.49 ±0.2a	13.2 ±3.5a
Guindilla	25 ±12a	5.8± 1.33a	7.7 ± 0.8b	0.17 ±0.1a	13.7±2.02a
Largo de Reus	60 ±18a	9.5 ±1.89a	12.2 ±1. 2a	0.56 ±0. 2a	22.3±2.86a
Blanco de Villena	48±12a	8.7± 1.33a	10.5 ±0. 8ab	0.14 ±0.1a	19.4±2.02a
Significance	NS	NS	*	NS	NS

*NS, *, ** and *** indicate not significant for a probability of $P > 0.05$ and significant for $P < 0.05$, 0.01 and 0.001, respectively, according to the F ratio (Student-Newman-Keuls test at a significance level of $p \leq 0.05$).*

Regarding the fully ripe stage, there was an increase in ascorbic acid content observed for the 'Bierzo', 'Largo de Reus' and 'Blanco de Villena'. 'Bierzo' showed 3.2-fold an increase compared to the green ripe stage. 'Largo de Reus' and 'Blanco de Villena' showed approximately 4-fold and 2.3-fold increments, respectively (Table 13). This observation aligns with previous studies that reported an increase in ascorbic content during pepper ripening (Zhang & Hamauzu, 2003), with vitamin C contents of 115.5 mg/100 g and 191.2 mg/100g FW in green and red peppers, respectively. Similar results were reported regarding the change in vitamin C

content during fruit ripening under organic farming conditions, showing an increase from 50.8 to 1488.5 FW in green and red ripe organic fruits (Jawad et al., 2013). Jawad et al. (2013) suggested that the increase in ascorbic content might be associated with its role of ascorbate as a photoprotector.

However, the content of ascorbic acid at dry weight condition (25 -77 mg/100 g DW) reported in the current study is significantly lower compared to the vitamin C content reported in previous works (Hallmann & Rembial kowska, 2012). In their study, 1976 mg/100g DW vitamin C content was reported for fully ripe fruits under organic farming conditions. Additionally, in the previous work of Zamljen et al. (2022), a vitamin C content of 1800 mg/100g at dry weight condition was reported in '*C. annuum* Cayenne' cultivar. In the present study, a significantly lower content of ascorbic acid was observed for variety Guindilla (25 mg/100 g DW) at fully ripe stage (Table 13), despite the lack of significant variety effect at the fully ripe stage. Previous studies have also reported a dramatical decrease in ascorbic acid concentration during drying and storage due to active antioxidation performance (Daood et al., 1996).

Sugar Content at Green Ripe and Fully Ripe Stages

Glucose and fructose were contributed more to total sugar both at green ripe (60% and 37.7 %) and fully ripe stage (56.7 % and 41.4 %), respectively. Meanwhile, the contribution of sucrose represented a lower amount, which is in agreement with a lower sucrose content of peppers reported in previous studies (Rosa-Martínez et al., 2021).

Concerning sugar content at green ripe stage, a significant difference among varieties was observed solely for sucrose content. Sucrose values ranged between 0.014 and 1.01 mg/100g DW for varieties Bola and Guindilla, respectively (Table 13). Non-significant variety effect was observed for fructose, glucose, and total sugar contents at green ripe stage.

The contribution of sucrose to total sugars was ranged from less than 1% to 6.9%, significantly lower compared to glucose and fructose. A decrease in sucrose content was observed with ripening, as indicated in previous works. Sucrose undergoes degradation during fruit maturation through an enzyme sucrose syntheses responsible for cleavage of sucrose during the late phase of growth in ripening fruits (Nielsen et al., 1991).

At the fully ripe stage, the average total sugar content increased considerably compared to the green ripe stage, except for the 'Guindilla' variety (Table 13). Total sugar content ranged between 13.2 g/100g and 22.3 g/100g DW for 'Bierzo' and 'Largo de Reus', respectively. Previous studies reported total sugar content of 44.8 g/100 g DW for *C. annuum* pericarp (Zamljen et al., 2021). Despite, no significant difference was observed for individual sugars fructose, sucrose and for total sugars at fully ripe stage, except for glucose. Variety 'Largo de Resus' showed significantly higher glucose content (12.2 mg/100g DW), compared to 'Guindilla (7.7 mg/100g DW), (Table 13). This range aligns with previous studies that reported a range of 7.8 g/100g to 11.0 g/100 g DW reported for fully ripe fruit under conventional farming conditions (Kim et al., 2019). The present study results are higher compared to a study focused on fruit composition profile of pepper, tomato and eggplant varieties, reporting a mean glucose content of pepper 30.4 g/kg FW for pepper (Rosa-Martínez et al., 2021). Moreover, a similar study including variety 'Bola' and 'Guindilla' at different ripening stage and growing conditions reported glucose content ranging from 7.5 to 38.5 g/kg FW during the fully ripe stage (Guijarro-Real et al., 2023).

Correlations between Main Flavonoids and Individual Sugars

Different degrees of correlations were observed among the main flavonoids -quercetin, luteolin, kaempferol and apigenin as well as within each individual sugar compound in relation to the main flavonoids at both green ripe and fully ripe stages (Figure 37). The highest positive correlation was found between luteolin and apigenin, as well as between quercetin and kaempferol, during the green ripe stage (Pearson product-moment correlation coefficient, p = 0.8).

Higher correlations were observed between quercetin and kaempferol, as well as between luteolin and apigenin, respectively, at the fully ripe stages (p = 0.9). These positive correlations among the main flavonoid components could be attributed to a concurrent accumulation, possibly due to shared transcriptional regulators of genes related to flavonoid biosynthesis (Kim et al., 2010).

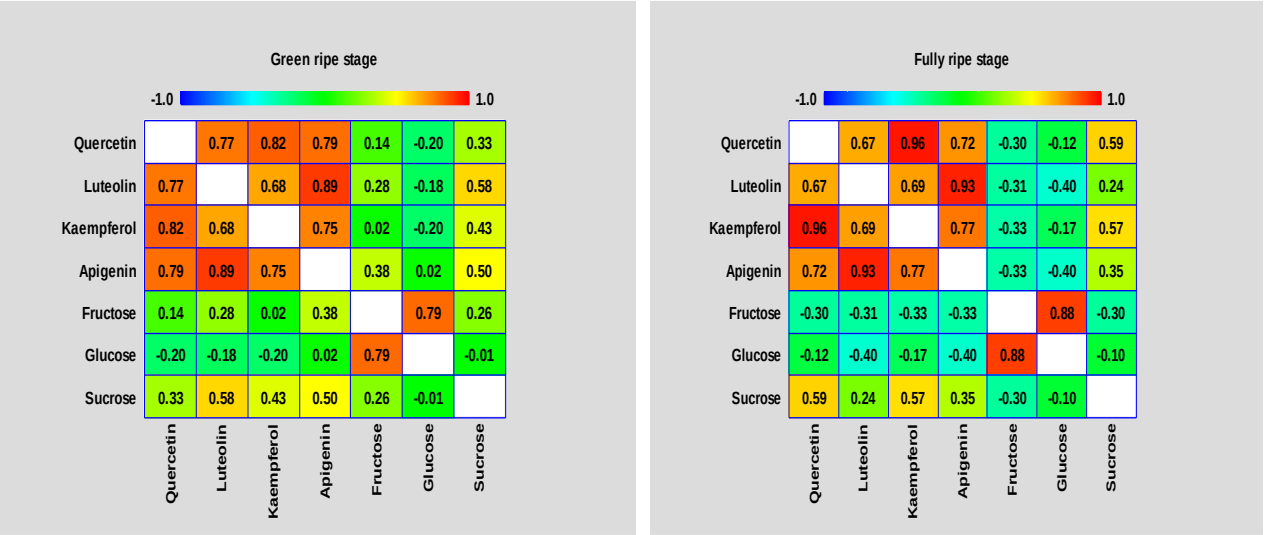


Figure 37. Pearson product-moment correlations for main flavonoids and individual sugar components at both green ripe and fully ripe stage.

In terms of correlations among sugar components, a higher positive correlation was observed between fructose and glucose, both at green ripe ($p = 0.79$) and fully ripe stage ($p = 0.88$) stages. Conversely, sucrose showed nearly zero correlation with fructose and glucose. On the contrary, an intermediate correlation ($p = 0.5$) was observed between sucrose and the main flavonoid components specifically, with luteolin and apigenin during the green ripe stage and with quercetin and kaempferol at the fully ripe stage. These positive correlations among the flavonoid components, as well as individual sugars, would allow indirect selection of each bioactive compounds according to the values of other correlated compounds.

5. CONCLUSIONS

These experimental findings highlight the contribution of organic farming to the phenotypic and nutritional qualities of locally adapted pepper varieties at different maturity stages. The tested widely adapted varieties exhibited variations in key fruit phenotypic traits, including fruit length, fruit diameter, weight, locules number, and total yield. Similarly, significant variations in bioactive compounds were observed within each variety at both ripening stages. The ripening stage also plays a significant role in the variation of these essential bioactive compounds.

The nutritional quality analysis for main flavonoids (Quercetin, Luteolin, Kaempferol, and Apigenin) and total phenolics demonstrated discernible effects both by variety and to some extent by ripening stage. Quercetin and luteolin accounted for a higher percentage of flavonoid component, 61% and 32%, respectively. While apigenin and kaempferol were present in relatively lower amounts. The ripening process increased the level of individual flavonoids _ luteolin, apigenin and kaempferol, while quercetin, total flavonoids and total phenolics decreased with ripening across all varieties. Notably, the variety 'Largo de Reus' bucked this trend with an increase in quercetin content at the fully ripe stage.

Ripening stage exerted an impact on individual sugars and total sugar content, with an increase was observed through ripening. However, variety 'Guindilla' diverged from this trend by displaying a decrease in sucrose and total sugars during ripening. Among individual sugars, glucose and fructose contributed a more prominent role in total sugar, 60% and 37.7 % at the green ripe stage and 57% and 41% at the fully ripe stage, respectively. While sucrose contributed significantly lower content for total sugars.

Carotenoid analysis was limited to ripe fruits, revealing a significant variety effect for red, yellow, and total carotenoid. Variety Bola exhibited the highest content of total carotenoids (3122 µg/g DW), as well as for red and yellow fractions, while the lowest value was recorded from variety Largo de Reus.

Correlation was detected within main flavonoid components, individual sugars, and between main flavonoids and sugar components at both ripening stages. Particularly noteworthy were correlations between quercetin and kaempferol, luteolin and apigenin among flavonoids, and a

correlation between fructose and glucose among individual sugars. These correlations would allow the potential for indirect positive selections.

Overall, the contents of major bioactive compounds in this organic agroforestry farming condition demonstrated higher values compared to previous studies. This underscores the significant contribution of organic farming, especially under agroforestry conditions, in enhancing the production of essential plant secondary metabolites. These compounds serve as defensive mechanisms against biotic and abiotic stress conditions and indirectly impact the nutritional quality attributes of pepper.

In conclusion, similar to previously reported studies, this study emphasizes the significance of organic farming, particularly under agroforestry conditions, as a sustainable and environmentally friendly approach. This approach aligns with the growing concern for food quality among the global population. To further solidify these compelling findings and considering the limitation of having data from only one year, it is recommended to repeat the experiment for an additional year to ensure consistency in the results.

6. REFERENCES

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877.

Arimboor, R., Natarajan, R. B., Menon, K. R., Chandrasekhar, L. P., & Moorkoth, V. (2015). Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: analysis and stability—a review. *Journal of Food Science and Technology*, *52*(3), 1258–1271.

Asnin, L., & Park, S. W. (2015). Isolation and Analysis of Bioactive Compounds in *Capsicum* Peppers. *Critical Reviews in Food Science and Nutrition*, *55*(2), 254–289.

Bae, H., Jayaprakasha, G. K., Jifon, J., & Patil, B. S. (2012). Extraction efficiency and validation of an HPLC method for flavonoid analysis in peppers. *Food Chemistry*, *130*(3), 751–758.

Baenas, N., Belović, M., Ilic, N., Moreno, D. A., & García-Viguera, C. (2019). Industrial use of pepper (*Capsicum annum L.*) derived products: Technological benefits and biological advantages. *Food Chemistry*, *274*, 872–885.

Baran Jha, T., Dafadar, A., & Ghorai, A. (2012). New genetic resource in *Capsicum L.* from Eastern Himalayas. *Plant Genetic Resources: Characterisation and Utilisation*, *10*(2), 141–144.

Daood, H. G., Vinkler, M., Márkus, F., Hebshi, E. A., & Biacs, P. A. (1996). Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. *Food Chemistry*, *55*(4), 365–372.

Devi, J., Sagar, V., Kaswan, V., Ranjan, J. K., Kumar, R., Mishra, G. P., Dubey, R. K., & Verma, R. K. (2021). Advances in Breeding Strategies of Bell Pepper (*Capsicum annuum L.* var. grossum Sendt.). In *Advances in Plant Breeding Strategies: Vegetable Crops*, *9*, 3–58.

Devi, M. (2017). Allelopathy in agroforestry: A review. *Journal of Pharmacognosy and Phytochemistry*, *6*(3), 686–688.

Dhatt, A. S., Sharma, M., & Kaur, B. (2020). Advances in Improvement of Pumpkin and Squashes. In *Accelerated Plant Breeding*, *2*, 301–335.

Do Rêgo, E. R., Finger, F. L., & do Rêgo, M. M. (2016). Production and breeding of chilli peppers (*capsicum* spp.). *Production and Breeding of Chilli Peppers* (*Capsicum* Spp.), 1–134.

Do Socorro Chagas, M. S., Behrens, M. D., Moragas-Tellis, C. J., Penedo, G. X. M., Silva, A. R., & Gonçalves-De-Albuquerque, C. F. (2022). Flavonols and Flavones as Potential anti-Inflammatory, Antioxidant, and Antibacterial Compounds. *Oxidative Medicine and Cellular Longevity*, *2022*, 21.

Du, J., Cullen, J. J., & Buettner, G. R. (2012). Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochimica et Biophysica Acta - Reviews on Cancer*, *1826*(2), 443–457.

Dubick, M. A., & Omaye, S. T. (2016). Grape wine and tea polyphenols in the modulation of

atherosclerosis and heart disease. In *Handbook of Nutraceuticals and Functional Foods, Second Edition*, 101–130.

Eshbaugh, W. H. (2012). The taxonomy of the genus *capsicum*. *Peppers: Botany, Production and Uses*, 14–28.

FAOSTAT (2023), https://www.fao.org/faostat/en/#data/QCL/visualize (accessed on, June 2023).

Feng, X., Yu, Q., Li, B., & Kan, J. (2022). Comparative analysis of carotenoids and metabolite characteristics in discolored red pepper and normal red pepper based on non-targeted metabolomics. *LWT*, *153*, 112398.

FIBL (2023), https://statistics.fibl.org/data.html (accessed on, June 2023).

Fratianni, F., D'acierno, A., Cozzolino, A., Spigno, P., Riccardi, R., Raimo, F., Pane, C., Zaccardelli, M., Lombardo, V. T., Tucci, M., Grillo, S., Coppola, R., & Nazzaro, F. (2020). Biochemical characterization of traditional varieties of sweet pepper (*Capsicum annuum L.*) of the campania region, southern italy. *Antioxidants*, *9*(6), 1–16.

Freitas, R. A. de, Nascimento, W. M., & Carvalho, S. I. C. de. (2008). Irrigação. In *Pimentas Capsicum*, Technical editor Flávia Aparecida Alcântara, 1st edition, 95–108.

Ghasemnezhad, M., Sherafati, M., & Payvast, G. A. (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *Journal of Functional Foods*, *3*(1), 44–49.

González-Pérez, S., Garcés-Claver, A., Mallor, C., Sáenz De Miera, L. E., Fayos, O., Pomar, F., Merino, F., & Silvar, C. (2014). New insights into Capsicum spp relatedness and the diversification process of *Capsicum annuum* in Spain. *PLOS ONE*, *9*(12), 1–23.

Guijarro-Real, C., Adalid-Martínez, A. M., Pires, C. K., Ribes-Moya, A. M., Fita, A., & Rodríguez-Burruezo, A. (2023). The Effect of the Varietal Type, Ripening Stage, and Growing Conditions on the Content and Profile of Sugars and Capsaicinoids in *Capsicum* Peppers. *Plants*, *12*(2), 231.

Ha, S. H., Kim, J. B., Park, J. S., Lee, S. W., & Cho, K. J. (2007). A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: Deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany*, *58*(12), 3135–3144.

Hallmann, E., & Rembial kowska, E. (2012). Characterisation of antioxidant compounds in sweet bell pepper (*Capsicum annuum L.*) under organic and conventional growing systems. *Journal of the Science of Food and Agriculture*, *92*(12), 2409–2415.

Hamed, M., Kalita, D., Bartolo, M. E., & Jayanty, S. S. (2019). Activities of *Capsicum annuum* : Comparative Study of the E ff ect of Ripening Stage and Cooking Methods. *Antioxidants*, *8*(364), 1–19.

Hernández-Pérez, T., Gómez-García, M. del R., Valverde, M. E., & Paredes-López, O. (2020). *Capsicum annuum* (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. *Comprehensive Reviews in*

Food Science and Food Safety, *19*(6), 2972–2993.

Hoesel, W., & Studies, E. (1979). Metabolism And Degradation Of Phenolic Compounds In Plant. *Biochemistry of plant phenolics*, 339–340.

Hornero-Méndez, D., Gómez-Ladrón de Guevara, R., & Minguez-Mosquera, M. I. (2000). Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. *Journal of Agricultural and Food Chemistry*, *48*(9), 3857–3864.

Hornero-Méndez, D., & Minguez-Mosquera, M. I. (2001). Rapid spectrophotometric determination of red and yellow isochromic carotenoid fractions in paprika and red pepper oleoresins. *Journal of Agricultural and Food Chemistry*, *49*(8), 3584–3588.

Howard, L. R., Smith, R. T., Wagner, A. B., Villalon, B., & Burns, E. E. (1994). Provitamin A and Ascorbic Acid Content of Fresh Pepper Cultivars (*Capsicum annuum*) and Processed Jalapeños. *Journal of Food Science*, *59*(2), 362–365.

Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry*, *48*(5), 1713–1720.

Howard, L. R., & Wildman, R. E. C. (2007). Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annuum*). *Handbook of Nutraceuticals and Functional Foods*, *165-191*.

Ipgri, A. (1995). CATIE. 1995. Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy; the Asian Vegetable Research and Development Center, Taipei, Taiwan, and the Centro Agronómico Tropical de Investigación y Enseñanza. *Turrialba, Costa Rica*, *17*(61), 4–68.

Jawad, M., Schoop, R., Suter, A., Klein, P., & Eccles, R. (2013). Perfil de eficacia y seguridad de Echinacea purpurea en la prevención de episodios de resfriado común: Estudio clínico aleatorizado, doble ciego y controlado con placebo. *Revista de Fitoterapia*, *13*(2), 125–135.

Jiménez, M., Escribano-Cebrián, J., & García-Carmona, F. (1998). Oxidation of the flavonol fisetin by polyphenol oxidase. *Biochimica et Biophysica Acta - General Subjects*, *1425*(3), 534–542.

Joshi, R., Punetha, P., & Singh, P. (2016). Agroforestry: A promising tool to mitigate climate change. In *Holistic Development of Agroforestry* , 159–166.

Jr, C. S., Mazourek, M., Stellari, G. M., Connell, M. O., & Jahn, M. (2007). *Genetic control of pungency in C . chinense via the Pun1 locus*. *58*(5), 979–991.

Kim, E. H., Lee, S. Y., Baek, D. Y., Park, S. Y., Lee, S. G., Ryu, T. H., Lee, S. K., Kang, H. J., Kwon, O. H., Kil, M., & Oh, S. W. (2019). A comparison of the nutrient composition and statistical profile in red pepper fruits (*Capsicums annuum* L.) based on genetic and environmental factors. *Applied Biological Chemistry*, *62*(1).

Kim, J. K., Lee, S. Y., Chu, S. M., Lim, S. H., Suh, S. C., Lee, Y. T., Cho, H. S., & Ha, S. H. (2010). Variation and correlation analysis of flavonoids and carotenoids in Korean

pigmented rice (*Oryza sativa* L.) cultivars. *Journal of Agricultural and Food Chemistry*, *58*(24), 12804–12809.

Kuhn, H., Padilha, M., & Lía Barbieri, R. (2016). Pages: 148-154 To Cite This Article: Henrique Kuhn Massot Padilha and Rosa Lía Barbieri., Plant breeding ofchili peppers (*Capsicum, Solanaceae*)-A review. *Australian Journal of Basic and Applied Sciences*, *10*(15), 148–154.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207–220.

Lee, Y., Howard, L. R., & Villalón, B. (1995). Flavonoids and Antioxidant Activity of Fresh Pepper (*Capsicum annuum*) Cultivars. *Journal of Food Science*, *60*(3), 473–476.

Lemos, V. C., Reimer, J. J., & Wormit, A. (2019). Color for life: Biosynthesis and distribution of phenolic compounds in pepper (*capsicum annuum*). *Agriculture (Switzerland)*, *9*(4), 1–29.

Liaaen-Jensen, S., & Andrewes, A. G. (1972). Microbial carotenoids. In *Annual review of microbiology*, *26*(1), 225-248.

Lone, A. H., & Rashid, I. (2023). Sustainability of Organic Farming *: A Review via Three Pillar Approach Sostenibilidad de la agricultura ecológica : una revisión a través del enfoque de tres pilares*, *11*.

Lufu, R., Ambaw, A., & Opara, U. L. (2019). The contribution of transpiration and respiration processes in the mass loss of pomegranate fruit (cv. Wonderful). *Postharvest Biology and Technology*, *157*, 110982.

Mac Rae, R., Juhasz, M., Langer, J., & Martin, R. (2006). Ontario Goes Organic: How to Access Canada's Growing Billion Dollar Market for Organic Food. *World Wildlife Fund and Organic Agriculture Centre of Canada*, *4*, 1–11.

Materska, M., & Perucka, I. (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry*, *53*(5), 1750–1756.

Mendes, N. D. S., Castello, É., & Andrade, B. De. (2020). Trends in Food Science & Technology The role of bioactive components found in peppers. *Trends in Food Science & Technology*, *99*, 229–243.

Merhan, O. (2017). The Biochemistry and Antioxidant Properties of Carotenoids. *Carotenoids*, *5*, 51.

Miean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, *49*(6), 3106-3112.

Mínguez-Mosquera, M. I., & Hornero-Méndez, D. (1994). Comparative Study of the Effect of Paprika Processing on the Carotenoids in Peppers (*Capsicum annuum*) of the Bola and Agridulce Varieties. *Journal of Agricultural and Food Chemistry*, *42*(7), 1555–1560.

Mohan Rao, A., & Anilkumar, C. (2020). Conventional and Contemporary Approaches to Enhance Efficiency in Breeding Chilli/Hot Pepper. In *Accelerated Plant Breeding*, *2* (2),

223–269.

Murthy, I. K., Dutta, S., Varghese, V., Joshi, P. P., & Kumar, P. (2017). Impact of Agroforestry Systems on Ecological Systems: A Review. *Global Journal of Science Frontier Research*, *16*(5), 15–28.

Navarro, J. M., Flores, P., Garrido, C., & Martinez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry*, *96*(1), 66–73.

Nielsen, T. H., Skjærbæ, H. C., & Karlsen, P. (1991). Carbohydrate metabolism during fruit development in sweet pepper (*Capsicum annuum*) plants. *Physiologia Plantarum*, *82*(2), 311–319.

Padilha, H. K. M., Pereira, E. dos S., Munhoz, P. C., Vizzotto, M., Valgas, R. A., & Barbieri, R. L. (2015). Genetic variability for synthesis of bioactive compounds in peppers (*Capsicum annuum*) from Brazil. *Food Science and Technology (Brazil)*, *35*(3), 516–523.

Perry, L., Dickau, R., Zarrillo, S., Holst, I., Pearsall, D. M., Piperno, D. R., Berman, M. J., Cooke, R. G., Rademaker, K., Ranere, A. J., Raymond, J. S., Sandweiss, D. H., Scaramelli, F., Tarble, K., & Zeidler, J. A. (2007). Starch fossils and the domestication and dispersal of chili peppers (*Capsicum* spp. L.) in the Americas. *Science*, *315*(5814), 986–988.

Pinheiro, P. F., & Justino, G. C. (2012). Structural analysis of flavonoids and related compounds-a review of spectroscopic applications. *Phytochemicals—A Global Perspective of Their Role in Nutrition and Health*, 33–56.

Putra, D. P., M, S. S., & Swasti, E. (2016). Pollination in chili pepper (*Capsicum annuum* L .) by *Trigona laeviceps* and *T . minangkabau*. *Journal of Entomology and Zoology Studies*, *4*(4), 191–194.

Re, G. A., Piluzza, G., Sanna, F., Molinu, M. G., & Sulas, L. (2019). Polyphenolic composition and antioxidant capacity of legume-based swards are affected by light intensity in a Mediterranean agroforestry system. *Journal of the Science of Food and Agriculture*, *99*(1), 191–198.

Ribes-Moya, A. M., Adalid-Martínez, A. M., Bracho-Gil, M., Esteve-Ciudad, P., Fernández-De-Córdova, P., Raigón, M. D., & Rodriguez-Burruezo, A. (2018). Effect of the Genotype and Growing Conditions on the Main Phenolic Compounds in *Capsicum* Peppers. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, *75*(1), 68.

Ribes-Moya, A. M., Adalid, A. M., Raigón, M. D., Hellín, P., Fita, A., & Rodríguez-Burruezo, A. (2020). Variation in flavonoids in a collection of peppers (*Capsicum* sp.) under organic and conventional cultivation: effect of the genotype, ripening stage, and growing system. *Journal of the Science of Food and Agriculture*, *100*(5), 2208–2223.

Ribes-Moya, A. M., Raigón, M. D., Moreno-Peris, E., Fita, A., & Rodríguez-Burruezo, A. (2018). Response to organic cultivation of heirloom *Capsicum* peppers: Variation in the level of bioactive compounds and effect of ripening. *PLoS ONE*, *13*(11), 1–24.

Rosa-Martínez, E., García-Martínez, M. D., Adalid-Martínez, A. M., Pereira-Dias, L., Casanova,

C., Soler, E., Figàs, M. R., Raigón, M. D., Plazas, M., Soler, S., & Prohens, J. (2021). Fruit composition profile of pepper, tomato and eggplant varieties grown under uniform conditions. *Food Research International*, *147*, 110531.

Ruiz, de G. J. I., Prohens, J., & Tierno, R. (2016). Pimiento. *Las variedades locales en la mejora genética de plantas*, Edited by Eusko Jaurlaritzaren Argitalpen Zerbitzu Nagusia Servicio Central de Publicaciones del Gobierno Vasco Donostia-San Sebastián, 405-426.

Sampaio, A. P. L., Aguilera, J. G., Mendes, A. M. da S., Argentel-Martínez, L., Zuffo, A. M., & Teodoro, P. E. (2023). The role of the genetic diversity of Capsicum spp. in the conservation of the species: Qualitative and quantitative characterization. *Ciencia e Agrotecnologia*, *47*.

Shaha, R. K., Rahman, S., & Asrul, A. (2013). Bioactive compounds in chilli peppers (*Capsicum annuum L* .) at various ripening (green , yellow and red) stages. *Annals of Biological Research*, *4*(8), 27–34.

Sharma, V. K., Srivastava, A., & Mangal, M. (2020). Recent Trends in Sweet Pepper Breeding. *Accelerated Plant Breeding, 2*, 417–444.

Shiau, E., Yap, P., Jitareerat, P., Uthairatanakij, A., Maier, C. S., & Laohakunjit, N. (2023). Targeted and untargeted metabolites and antioxidant properties in chili pepper at different maturity stages. *Crop Science*, *63*(4), 2405–2416.

Siddique, S., Hamid, M., Tariq, A., & Kazi, A. G. (2014). Organic Farming : The Return to Nature. *Improvement of crops in the Era of Climatic Changes, 2*, 249–281.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. B. T.-M. in E. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *In Methods in enzymology* 299, 152–178.

Sricharoen, P., Lamaiphan, N., Patthawaro, P., Limchoowong, N., Techawongstien, S., & Chanthai, S. (2017). Phytochemicals in *Capsicum* oleoresin from different varieties of hot chilli peppers with their antidiabetic and antioxidant activities due to some phenolic compounds. *Ultrasonics Sonochemistry*, *38*, 629–639.

Starke Ayres. (2019). *Sweet-Hot-Pepper-Production-Guideline-2019*.

Swammy, K. R. M. (2023). *Origin , Distribution , Taxonomy , Botanical Description , Genetic Diversity*. *13*(03), 61956–61977.

Swapnil, P., Meena, M., Singh, S. K., Dhuldhaj, U. P., Harish, & Marwal, A. (2021). Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Current Plant Biology*, *26*, 100203.

Szafirowska, A., & Elkner, K. (2009). The comparison of yielding and nutritive value of organic and conventional pepper fruits. *Vegetable Crops Research Bulletin*, *71*(1), 111–121.

Thangaraja, A. (2022). A study on consumers problems and prospects of organic food products in special reference, *European Journal of Molecular & Clinical*, *09*(08), 3281–3288.

Thuphairo, K., Sornchan, P., & Suttisansanee, U. (2019). Bioactive compounds, antioxidant

activity and inhibition of key enzymes relevant to Alzheimer's disease from sweet pepper (*Capsicum annuum*) extracts. *Preventive Nutrition and Food Science*, *24*(3), 327–337.

Topuz, A., & Ozdemir, F. (2007). Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. *Journal of Food Composition and Analysis*, *20*(7), 596–602.

Vázquez-Espinosa, M., González-de-Peredo, A. V., Espada-Bellido, E., Ferreiro-González, M., Barbero, G. F., & Palma, M. (2023). The effect of ripening on the capsaicinoids composition of Jeromin pepper (*Capsicum annuum* L.) at two different stages of plant maturity. *Food Chemistry*, *399*, 133979.

Welbaum, G. E. (2015). Family Solanaceae. In *Vegetable production and practices*, 176–221.

Wiedenfeld, R. (2019). Onion Growth and Nutrient Uptake Patterns As Affected By Nitrogen Rate and Timing of Application. In *HortScience* 27 (11), 1173.

Willer, H., Schlatter, B., & Trávní, J. (2023). *The World of Organic Agriculture Statistics and Emerging Trends 2023*, Edited by Helga Willer, Bernhard Schlatter and Jan Trávníček, Germany, 148–163.

Young, J. E., Zhao, X., Carey, E. E., Welti, R., Yang, S. S., & Wang, W. (2005). Phytochemical phenolics in organically grown vegetables. *Molecular Nutrition and Food Research*, *49*(12), 1136–1142.

Zamljen, T., Jakopič, J., Hudina, M., Veberič, R., & Slatnar, A. (2021). Influence of intra and inter species variation in chilies (*Capsicum* spp.) on metabolite composition of three fruit segments. *Scientific Reports*, *11*(1), 1–11.

Zamljen, T., Medič, A., Veberič, R., Hudina, M., Jakopič, J., & Slatnar, A. (2022). Metabolic variation among fruits of different chili cultivars (*Capsicum* spp.) using HPLC/MS. *Plants*, *11*(1), 101.

Zhang, D., & Hamauzu, Y. (2003). Phenolic compounds , ascorbic acid , carotenoids and antioxidant properties of green , red and yellow bell peppers. *Journal of Food, Agriculture and Environment*, *1*(2), 22–27.