REDISCOVERING PEPPER, EGGPLANT AND LETTUCE LANDRACES OF THE VALENCIAN COMMUNITY; AN ANCIENT RESOURCE WITH VAST POTENTIAL FOR THE FUTURE.

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RESUMEN

La erosión genética provocada en los cultivos al primar producción sobre calidad ha derivado en pérdida de biodiversidad, lo que compromete la seguridad alimentaria mundial. Los agricultores, a través de un proceso histórico de selección, han ido diferenciando variedades tradicionales de cultivo que sonfuente de biodiversidad agrícola que además favorece el desarrollo de la economía local. Por este motivo, su recuperación, clasificación y cultivo son clave para la economía y futuro alimentario. La conservación de las variedades tradicionales requiere un conocimiento de las mismas a través de la descripción detallada de las características fenotípicas, agronómicas, y de calidad nutricional como valor añadido. La Comunitat Valenciana, cuenta con un extenso patrimonio hortícola constituido por una gran diversidad de variedades tradicionales de hortalizas. Estas son fruto de la adaptación a variadas condiciones agroclimáticas de la geografía valenciana, por un lado, y de la selección aplicada por los agricultores en cada localidad por otro. En este sentido, estas variedades tienen un gran valor como patrimonio etnobotánico y como tal deberían ser conservadas. Asimismo, en la actualidad, el cultivo y el consumo de las variedades tradicionales están creciendo, ya que son especialmente atractivas para los consumidores por su diversidad y su alta calidad nutracéutica.

En este contexto, esta tesis doctoral se basa en la caracterización fenotípica y nutricional para valorizar las variedades tradicionales de la comunidad, correspondientes a los cultivos de pimiento, berenjena y lechuga, con la finalidad de promover su conservación y cultivo en las zonas de origen, e impulsando la diversidad.

La caracterización morfológica de las variedades autóctonas ha sido objeto de numerosos estudios, necesarios porque proporcionan información sobre los caracteres fenotípicos diferenciadores, y contribuyen a optimizar los programas de mejora vegetal. En este sentido, la caracterización de las variedades hortícolas valencianas seleccionadas se realizó siguiendo las directrices del IBPGR. Además, en esta tesis doctoral se han realizado estudios sobre el valor nutracéutico de las tres variedades seleccionadas por ser uno de los principales intereses del consumidor. Por ello, el contenido de algunos compuestos bioactivos y antioxidantes (fenoles, flavonoides, antocianinas, ácido ascórbico, licopeno, carotenoides, clorofilas y la actividad antioxidante), azucares y minerales fueron monitoreados para establecer parámetros de calidad en las especies mencionadas. También se determinó los parámetros indicativos de estrés oxidativo, para establecer la capacidad de conservación de atributos físico-químicos de la lechuga en el ensayo de post-cosecha.

ABSTRACT

Genetic erosion in crops, gained from prioritising production over quality, has led to biodiversity loss, which compromises global food security. By a historic selection process, farmers have been differentiating traditional crop varieties, which are a source of agricultural biodiversity that also favours the development of local economy, which makes their recovery, classification and cultivation key for food economy and the future. The conservation of traditional varieties requires knowledge of them, obtained from a detailed description of their phenotypical, agronomic and nutritional quality characteristics as added value. The Valencian Community (east Spain) has extensive horticultural heritage that is made up of a high diversity of traditional vegetable varieties. These are the result of adapting to the varied agroclimate conditions of the Valencian geography: on the one hand, the selection applied by farmers to each locality; on the other hand, these varieties are very valuable as ethnobotanical heritage and should be preserved. Moreover, the cultivation and consumption of traditional varieties are currently growing because they are particularly appealing to consumers for their diversity and high nutraceutical quality.

In this context, the present doctoral thesis is based on a phenotypical and nutritional characterisation to evaluate traditional varieties in the Valencian Community, which correspond to pepper, eggplant and lettuce crops, to promote their conservation and cultivation in areas of origin, and to boost diversity.

The morphological characterisation of landraces has been the subject of many studies, which are necessary because they provide information about differentiating phenotypical characteristics and help to optimise plant-breeding programmes. The characterisation of the selected Valencian vegetable varieties was carried out following IBPGR guidelines. Furthermore, studies were conducted in this doctoral thesis into the nutraceutical value of the three selected crops because this value is one of the main consumer interests. The content of some bioactive compounds and antioxidants (phenols, flavonoids, anthocyanins, ascorbic acid, lycopene, carotenoids, chlorophylls, antioxidant activity), sugars and minerals were monitored to establish quality parameters in the aforementioned species. Parameters indicative of oxidative stress were also determined to establish the conservation capacity of the physico-chemical attributes of lettuce in the post-harvest test.

RESUM

L'erosió genètica provocada en els cultius com a conseqüència de posar per davant producció sobre qualitat ha derivat en pèrdua de biodiversitat, fet que compromet la seguretat alimentària mundial. Els agricultors, a través d'un procés històric de selecció, han generat la diferenciació varietats tradicionals de cultiu que hui són font de biodiversitat agrícola. A més, s'afavoreix el desenvolupament de l'economia local. Per aquest motiu, la seva recuperació, classificació i cultiu són clau per a l'economia i el futur alimentari. La conservació de les varietats tradicionals requereix un coneixement de les mateixes mitjançant la descripció detallada de les seues característiques fenotípiques, agronòmiques, i de qualitat nutricional com a valor afegit. La Comunitat Valenciana compta amb un extens patrimoni hortícola constituït per una gran diversitat de varietats tradicionals d'hortalisses. Aquestes són fruit de l'adaptació a diverses condicions agroclimàtiques de la geografia valenciana, d'una banda, i de la selecció aplicada pels agricultors a cada localitat de l'altra. En aquest sentit, aquestes varietats tenen un gran valor com a patrimoni etnobotànic i com a tal haurien de ser conservades. Així mateix, actualment, el cultiu i el consum de les varietats tradicionals estan creixent, ja que són especialment atractives per als consumidors per la seua diversitat i la seua alta qualitat nutracèutica.

En aquest context, aquesta tesi doctoral es basa en la caracterització fenotípica i nutricional per valoritzar les varietats tradicionals de la Ccomunitat, corresponents als cultius de pebre, albergínia i encisam, amb la finalitat de promoure'n la conservació i el cultiu a les zones d'origen, i impulsant la diversitat.

La caracterització morfològica de les varietats autòctones ha estat objecte de nombrosos estudis, necessaris perquè proporcionen informació sobre els caràcters fenotípics diferenciadors, i contribueixen a optimitzar els programes de millora vegetal. En aquesta línia, la caracterització de les varietats hortícoles valencianes seleccionades es va fer seguint les directrius de l'IBPGR. A més, en aquesta tesi doctoral s'han fet estudis sobre el valor nutracèutic de les tres espècies seleccionades per ser un dels principals interessos del consumidor. Per això, el contingut d'alguns compostos bioactius i antioxidants (fenols, flavonoides, antocianinas, àcid ascòrbic, licopè, carotenoides, clorofil·les i l'activitat antioxidant), sucres i minerals van ser monitoritzats per establir paràmetres de qualitat a les espècies esmentades. També es van determinar els paràmetres indicatius d'estrès oxidatiu, per establir la capacitat de conservació d'atributs fisicoquímics de l'encisam a l'assaig de postcollita.

LABURPENA

Ekoizpena kalitatearen gainetik lehenesteak eta laboreetan eragindako higadura genetikoak biodibertsitatearen galera eragin du, eta horrek munduko elikagaien segurtasuna arriskuan jartzen du. Nekazariak, hautaketa prozesu historiko baten bidez, barietate tradizionalak bereizten joan dira nekazaritzako biodibertsitate iturri diren eta tokiko ekonomiaren garapena ere faboratzen duten horiek. Hori dela eta, haren berreskurapena, sailkapena eta laborantza funtsezkoak dira elikagaien ekonomiarako eta etorkizunerako. Barietate tradizionalak kontserbatzeko ezaugarri fenotipiko, agronomiko eta nutrizio-kalitatearen deskribapen zehatzaren bidez ezagutzea eskatzen du. Valentziako Erkidegoak barazki-barietate tradizionalen aniztasun handiz osaturiko baratze-ondare zabala du. Alde batetik, Valentziako geografiako baldintza agroklimatiko anitzetara egokitzearen ondorio dira. Bestetik, herri bakoitzean nekazariek aplikatzen duten hautapenaren ondorioa. Zentzu honetan, barietate hauek ondare etnobotaniko gisa balio handia dute, horrenbestez, gorde egin behar dira. Era berean, gaur egun, barietate tradizionalen laborantza eta kontsumoa hazten ari dira, kontsumitzaileentzat bereziki erakargarriak baitira aniztasunagatik eta kalitate nutrazeutiko handiagatik.

Testuinguru honetan, doktoretza hau ezaugarri fenotipiko eta nutrizionaletan oinarritzen da. Komunitateko barietate tradizionalak balioesteko, piper, berenjena eta letxuga laboreen kontserbazioa eta jatorriko eremuetan haztea sustatzeko helburuarekin zuzenduta dago, aniztasuna sustatzearekin batera.

Bertako barietateen karakterizazio morfologikoa ikerketa ugari egin da, beharrezko ezaugarri fenotipiko bereizgarriei buruzko informazioa ematen dutelako, eta landareak ugaltzeko programak optimizatzen laguntzen dutelako. Zentzu honetan, hautatutako Valentziako baratze barietateen karakterizazioa IBPGRren jarraibideei jarraituz egin da. Gainera, doktoretza honetan kontsumitzailearen interes nagusietako bat izateagatik aukeratutako hiru espezieen balio nutrazeutikoari buruzko azterketak egin dira. Hori dela eta, konposatu bioaktibo eta antioxidatzaile batzuen edukia (fenolak, flavonoideak, antozianinak, azido askorbikoa, likopenoa, karotenoideak, klorofilak eta jarduera antioxidatzailea), azukre eta mineralen edukia kontrolatzen da, aipatutako espezieetan kalitate-parametroak ezartzeko. Estres oxidatiboaren parametroak ere zehaztu dira uzta osteko saiakuntzan, letxugaren ezaugarri fisiko-kimikoen kontserbazio ahalmena ezartzeko.

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CHAPTER 01 INTRODUCTION

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INTRODUCTION

1. TRADITIONAL VARIETIES

1.1. Definition and general characteristics

Traditional varieties, or **landraces**, are dynamic and genetically diverse populations of historic origin and distinct identity that are adapted to a specific geographical area and are normally associated with traditional farming practices [1,2]. These varieties are recognised by farmers thanks to their characteristic morphology. As a general rule, they are usually named according to their local origin, a geographical area to which they are perfectly adapted. These geolocal conditions are defined by **abiotic** (salinity, drought, temperature, etc.), **biotic** (diseases, pests, weeds, etc.) and **human** (cultivation, management and use) factors [3]. For this reason, it is understood that landraces are **adapted** to the type of agriculture in the area (soil type, sowing type, maturity date, attitude, among other properties) [4], and are also able to effectively respond to changes in the environment [5]. This implies that they possess a kind of built-in insurance against environmental risks, possibly due to their inherited population structure [4], and for having accumulated **resistance genes** that allow them to cope with the physico-biological factors in that specific environment [3]. For this reason, they are capable of producing **benefits** for farmers, albeit at a self-sufficiency level, to face possible environmental disasters [3]. So although they are varieties that are generally not noted for their high productivity, they are stated to be **stable yielding** crops, but adaptable ones to local climate change scenarios [3,6].

1.2. Origin of landraces

Although is still a controversial concept, landraces have a relatively long selection and cultivation history, which is certainly much longer than short-lived modern cultivars. No consensus has been reached about how traditional varieties have been cultivated. Several authors suggest that they have been grown "since time immemorial" [7], "for long periods of time" [8], "for hundreds, and even thousands of years" [9], "over a long period of time" [10]. Very few, however, explicitly indicate how long a landrace must be cultivated to indeed be considered a local variety [1].

Landraces were originally defined as varieties that had been cultivated in a **particular locality** for a long time (without specifying how long), and they had adapted to local growing conditions through natural selection, usually without farmers' intentional selection [6]. Many authors associate landraces with the total lack of human selection by, thus, assuming that their appearance is entirely and exclusively dependent on nature, and is mediated by time and natural selection [3]. In contrast, other authors suggest that human selection has occurred, but in the form of unconscious selection or with some degree of awareness [3], and suggest that landraces **lack "formal" genetic improvement** [3]. So their evolution would have been based on both natural conditions and human activity [2]. This last opinion would be the most accurate one based on specific and striking landrace characteristics, which makes them appealing for both farmers and consumers.

The way in which traditional varieties have been selected has enabled the high degree of **variability** among them. This heterogeneity is responsible for the enormous plasticity shown by traditional varieties under stress conditions,

and it is found at both **inter- and intrapopulation levels** [11]. During the selection and differentiation process of traditional varieties, special attention has been paid to those variants with the best **organoleptic characteristics [**11]. It is, therefore, known that these local varieties are highly **nutritious** [3,12]. Furthermore, the initial domestication from wild parents to cultivars that can be grown, harvested and consumed represents intense measured selection by farmers [13]. As previously mentioned, the most important agronomic characteristic of a landrace is its **yield stability**. Farmers have related the level of variation to the importance of this trait and have consciously promoted or created variation [6] by turning to agricultural biodiversity and seed production, which are indispensable factors in agriculture [14]. In this way, traditional agricultural production systems in the past have played a key role in the evolution and conservation of on-farm diversity by allowing farmers to avoid crop failure by reducing vulnerability to environmental stresses [3].

This combination of human and environmental selection continues due to farmers' interest in selection that favours the varieties of most interest for them, while the **environment selects** the traits that increase fitness [15]. The introduction of new commercial or local varieties is often linked with farmers' need to cope with environmental constraints in a constantly changing climate [13]. Local community continuity can be maintained through farmers' seed exchange networks. Indeed several papers have highlighted the importance of seed exchange for maintaining local varieties [3]. Zeven [6] suggests that landrace diversity can be explained by the combination of farmers' selection criteria and specific local genotypes through farmers' seed saving and introducing variations by exchanging other genotypes of the same crop with other farmers. In this context, **gene flow** through **cross-pollination and seed exchange** drives variation and recombination, along with ultimate population differentiation. In turn, **natural mutations** also increase the degree of diversity [15]. Taken together, it is suggested that the genetic diversity of these landraces is continuously remodelled [15].

1.3. Genetic erosion

The emergence of agriculture about 10,000 years ago altered the ecological balance of many systems. Fortunately during this **domestication** process, of the more than 300,000 flowering plants to have been described, humans have adapted more than 7,000 species to meet basic human needs [16].

During the period from 800 to 1500 (the Middle Ages), many human activities shifted from rural areas to urban centres. The urban population's **food requirements** grew, which resulted in a higher demand for agriculture [17]. The population concentration in urban areas and increasing food demands created a situation in which high production based on **uniform crops** was prioritised over more reliable and diversified production [16].

Initially, the most marked advances were made by farmers who improved cultivation practices, and who selected seeds that would produce higher yielding crops by experimenting with breeding and newly acquired crops. Indeed they became professional food producers [17]. Furthermore, technological change during the Agricultural Revolution in the 1760s transformed agriculture through the productivity of the countryside by decoupling farming areas from urban areas. Thus while facilitating urbanisation, together with continuously rising populations and the reduction of cultivated areas, the intensification of farming practices was further promoted [17]. Halfway through the 19th

century, the first seed companies began to emerge and formal breeding programmers were born with them. In the mid-20th century, seed houses controlled the market, and most farmers preferred to grow improved crops rather than the traditional varieties that they had always grown. Consequently, the original agricultural objective based on self-sufficiency, which ruled until the 1960s, had fallen into disuse by the 1980s. The production levels needed to feed the European population could be met by a fewer farmers, and the cultivated areas managed by each farmer were much larger [1]. This was driven by a major revolution in the seed supply systems of improved vegetable materials [11]. In the 1990s, only 5% of the population, who lived in industrialised areas, was capable of generating enough food for the entire population, as opposed to the 70-80% required 300 years ago [17]. In this context, it can be stated that two factors have conditioned crop evolution processes: a) population increase; b) spatial integration of production systems [18]. Production diversity gave way to monoculture or livestock specialisation. Farms became highly capitalised and replaced human labour with technology, which contributed to rural exodus [14].

Likewise in the 1980s, the globalisation of markets also led to the globalisation of crop pests and diseases. Commercial varieties evolved and incorporated genes to resist these biotic agents [11]. In contrast, traditional varieties were adapted to the specific biotic stresses of the geographical area in which they grew, but did not show tolerance or resistance to the newly introduced pathogens [11]. The rise in modern agriculture was, therefore, linked with the loss of a number of plant species on which humans depended for food, which affected both semidomesticated wild ancestors and traditional cultivated varieties, even though they were crops that had enhanced food security in the past [13]. Thus it could be argued that breeding itself has been the main cause of diversity loss in traditional varieties. This phenomenon is known as the **"breeder's paradox"** [14].

Today, barely more than 150 species are cultivated, and most of humanity lives on no more than 12 plant species [16]. With very few exceptions, both public and private sectors invest in research into the production of global vegetable hybrids (lettuce, tomatoes, onions, etc.), while local varieties are neglected [13]. Furthermore, the intensification of horticultural crops requires developing varieties of stable production to face climate changes, and are adaptable to diverse agro-ecosystems [13]. Of the main crop species, a limited number of high-yielding standard varieties has been developed [16]. Unfortunately, at the same time, these practices have brought about loss of innumerable heterogeneous landraces.

Landraces have generally been replaced with market-driven production, mainly due to their low production rate, worse resistance to pests and diseases, and shorter postharvest life than commercial varieties [19]. However, landraces particularly constitute a valuable gene pool of increased diversity that can be exploited in breeding programmes to produce new commercial cultivars with specific traits [20]. Loss of species and **landraces** often leads to the irreversible **loss of the genetic diversity** that they contain, which is known as genetic erosion [16]. It is true that the disappearance of a given landrace does not imply the irretrievable loss of certain genes, but each traditional variety constitutes a unique combination of genes. So once one is lost, it is very difficult to recover it through a breeding programme [11,21]. For this reason, and contrarily to the notion that landraces would inevitably disappear (1960s) [6], they continue to play an important role in agricultural production today, especially in marginal environments where cultivars lose their competitive advantage [2].

1.4. Traditional varieties in modern agriculture

1.4.1 Preserving plant genetic resources; a demand for maintaining biodiversity

Today, the **nutritional safety** concept has become the **essential food security element**, and nutritional diversity is the basic component to ensure the human population's health [2]. The genetic diversity of crucial crops for feeding humanity, for the environment and for sustainable development is being dramatically lost [16].
The conservation and sustainable use of genetic resources go far beyond preventing species extinction. Plant genetic resources can be conserved ex situ in, for example, gene banks (facilities that store samples), accessions of crop genetic diversity, usually as seeds and vegetative material, or in situ either on farms with farmers' varieties or in nature reserves or protected areas with wild plants [22]

Concern about maintaining biodiversity emerged at the end of the last century as one of the strategic objectives for our planet's future [14]. Thousands of samples are now stored in germplasm banks around the world. In addition, although intensive agriculture dominates on a large scale, the cultivation of landraces is still common in many parts of the world, especially in in crop centres, and in origin or diversity terms [15].

In this context, the Intergovernmental Commission on Genetic Resources for Food and Agriculture (CGRFA), established by the Food and Agriculture Organization (FAO) of United Nations, was set up by the FAO in 1983 [23]. This Commission is a permanent forum for governments to discuss and negotiate issues of concern about genetic resources for food and agriculture. Similarly, the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) was adopted by the FAO in 2001 and it came into force in 2004. Its main objectives are the conservation and sustainable use of plant genetic resources for food and agriculture, and the fair and equitable sharing of the benefits that arise from their use. All this is in harmony with the Convention on Biological Diversity (CBD) for sustainable agriculture and food security [23]. Likewise, the Globally Important Agricultural Heritage Systems Initiative (GIAHS) takes an integrated approach to in situ conservation, which aims to promote the conservation and sustainable management of the world's most important indigenous and traditional farming systems and their associated biological and cultural diversity [16].

Today, both **in situ and ex situ** conservation methods are considered complementary, and the development of appropriate national and international strategies for their effective use is required [15,16]. In fact both the CBD and the Second Global Plan of Action for PGRFA [24], as well as Sustainable Development Goal 2.5 [25], have identified the ex situ and in situ conservation of plant genetic resources as critical and complementary activities. Furthermore, the voluntary guidelines for the conservation and sustainable use of landraces [26] also indicate that the in situ conservation of landraces should be complementary to ex situ conservation. According to the PGRFA, approximately 7.4 million genotypes (germplasm sources belonging to more than 16,500 plant species) are currently stored in 1,750 gene banks and collections worldwide [2]. About 1 million accessions of crops used wholly or partially as vegetables are conserved ex situ. Strictly speaking for the crops used exclusively as vegetables, about 518,000 vegetable accessions are conserved ex situ, which represent 7% of the 7.4 million PGRFA accessions worldwide [13].

Thus traditional varieties constitute a valuable reservoir of genetic diversity, which can be exploited for both a) breeding programmes to obtain new commercial genotypes with specific traits; b) a source of germplasm to reverse loss of variability [2,3,27]. Specifically, the greatest contribution of landraces for plant breeding is associated with the possession of traits related to more efficient absorption and usage of nutrients. They are also carriers of genes related to resistance to unfavourable environments due to water scarcity, salinity or high temperatures and biotic stresses [3]. Landraces are also known for being highly nutritious [3,12] because they were selected according to their organoleptic properties [11]. All these attributes are key reasons why landrace conservation and use should be intensified.

1.4.2 Public perception of traditional varieties; today's consumer demand

The growth of global landrace consumption, associated with quality products generated in traditional farming systems detected in recent years, has significantly increased food sales because consumer demand for natural, local and high-quality produce is also growing [28]. This new paradigm has also stimulated new market strategies [29,30], expanded market niches and created **new demands** [12,28,31]. This market type is known as a quality market. So although traditional varieties do not offer high-yield rates like modern varieties, they compensate their lower production with a higher price paid for product and flavour. It is in this reality where organoleptic and functional qualities converge, and where the importance of traditional varieties is framed. It is easy to understand why traditional varieties have continued to be cultivated, which now occupy a commercial niche to meet the needs of customers who prefer buying higher quality products, even if it means paying a higher price [32].

For many consumers, the term "local" is associated with small and environmentally eco-friendly farms. However, it should be noted that where food is produced does not necessarily guarantee the ecological sustainability or lack of environmental impacts triggered by the production system [29]. Likewise, the term "local" supports the **local food** movement as a "collaborative effort to build a more local, food self-sufficient economy". Sustainable food production, processing, distribution and consumption are integrated to improve the economy, environment and society of a particular place [12]. Thus the local food concept encompasses preferences for purchasing locally produced goods and services, rather than those produced by corporate institutions located far from purchasing places. So it is not only a geographical concept, but is also defined in terms of the food supply chain characteristics and its social impact [29]. The term "Local food" has several possible definitions, but also incites disagreement. In most cases, it implies food that has been grown in the vicinity (a few kilometres away, in the city itself, in the state itself, etc.) [33]. It can also be used to refer to food sold on alternative markets [29] and to food that is unique to a particular locality, or simply that is tied to a local meaning and value [34]. For all these reasons, certification and origin protection schemes in the EU focus research on the region of origin and the analysis of consumer preferences for regional foods [29]. In this context, landraces are gaining interest on specific markets characterised by placing an emphasis on local production, organic farming and consumer demand [27,28,35], and for having evolved under low-input conditions, similarly to those required in today's organic farming [20]. Therefore, it is crucial to promote studies that aim to **recover traditional varieties**, including phenotyping platforms for farmers and consumers if possible [28].

2. FOOD QUALITY

2.1. Organoleptic quality; the physico-chemical basis of fruit and vegetable quality

The food quality concept is wide, subjective and variable over time [11]. It covers several aspects like external appearance, nutritional value, presence of health-related compounds, safety and security [36]. It is related to the ability to meet different users and consumers' demands and to live up to their expectations. Food quality can be defined as a set of those characteristics that differentiate individual units of a product and are important for determining the degree of acceptability of that unit for users [37]. In this context, the quality definition varies depending on the point of view of the person concerned: a) farmers emphasise the importance of yield, easy handling and resistance to pests and diseases; b) processors value a product's homogeneity, the necessary technological requirements and the achieved processing rates; c) commercial chain agents essentially value visual appearance, product uniformity and a variety's postharvest performance; d) consumers pay attention to external product appearance, its organoleptic and functional quality, its freshness, its origin, the followed production system, and other aspects like its traditional use [11]. This means that the quality product term is not universal. However, consumer tastes have generally changed recently, which has enabled the market share of traditional varieties to increase.

As far as end consumers are concerned, purchase attributes (size, colour, firmness, aroma and no visual defects) and consumption (mainly taste and texture) stand out for judging quality [37,38]. As they are considered the main criteria for determining immediate quality, they are used as quality indicators throughout the supply chain, from farms to consumers, and ultimately determine product acceptance or rejection [39], despite it being difficult to quantify the attributes that drive acceptance or rejection [40]. Consumers evaluate these quality attributes, and consciously or unconsciously assign them a score to "mentally calculate" an overall quality score for purchase decisions [37]. The evaluation of quality attributes is, thus, an essential component of quality appreciation [41]. Consumer orientation to quality requires an understanding of consumer behaviour and focuses on predicting product performance in the marketplace. So the studies that measure consumer attitudes can be simplified to determine acceptability or willingness to buy at purchasing places and for consumption purposes [40]. By means of combining quantitative consumer panels and descriptive sensory analysis, it is possible to verify or refute what consumers claim about critical quality attributes [40]. Furthermore, nutritional quality is a parameter that is often neglected as a quality attribute when making food choices and purchasing decisions, and one that is impossible to see, taste or feel, even though it is an extremely important quality component [37,42]. For this reason, some believe that the **"quality"** concept should only include measurable and quantifiable product characteristics (including nutritional parameters), and consumer perceptions and responses to these characteristics should be called **"acceptability"** [11]. However, different views are also taken on the quality of fresh vegetables. According to Rouphael [43], quality has a dimension that depends on both the product and consumer.

2.2. Benefits that derive from consuming fruit and vegetables as nutritionally rich products

Plant foods contain almost all the essential nutritional compounds for human nutrition, as well as a large number

of **health-promoting** chemicals [44,45]. Although plant metabolite levels are affected by genetic and environmental factors, and by transport and storage conditions (Hounsome, 2008), they are considered major sources of vitamins (C, A, B1, B1, B6, B9, E), minerals, dietary fibre and phytochemicals [46]. Fortunately, they form part of our routine diet, but are relative to geographical distribution and cultivation [47]. Although international trade has increased the availability and scope of cultivated varieties, according to the 2007 World Health Report, unbalanced diets with low intakes of vegetables, complex carbohydrates and dietary fibre cause an estimated 2.7 million deaths per year, and are one of the top 10 risk factors that contribute to mortality [46]. Indeed the World Health Organisation (WHO) recommends a daily intake of 400 g of edible fruit and vegetables to prevent non-communicable diseases, and to avoid and alleviate various micronutrient deficiencies [13]. In fact increased vegetable consumption has been linked with a 15% reduction in the cancer risk, a 30% reduction in the cardiovascular disease risk and a 20% reduction in mortality. These benefits are attributed to the intake of antioxidants, such as ascorbic acid, vitamin E, carotenoids, lycopenes, polyphenols and other phytochemicals [48].

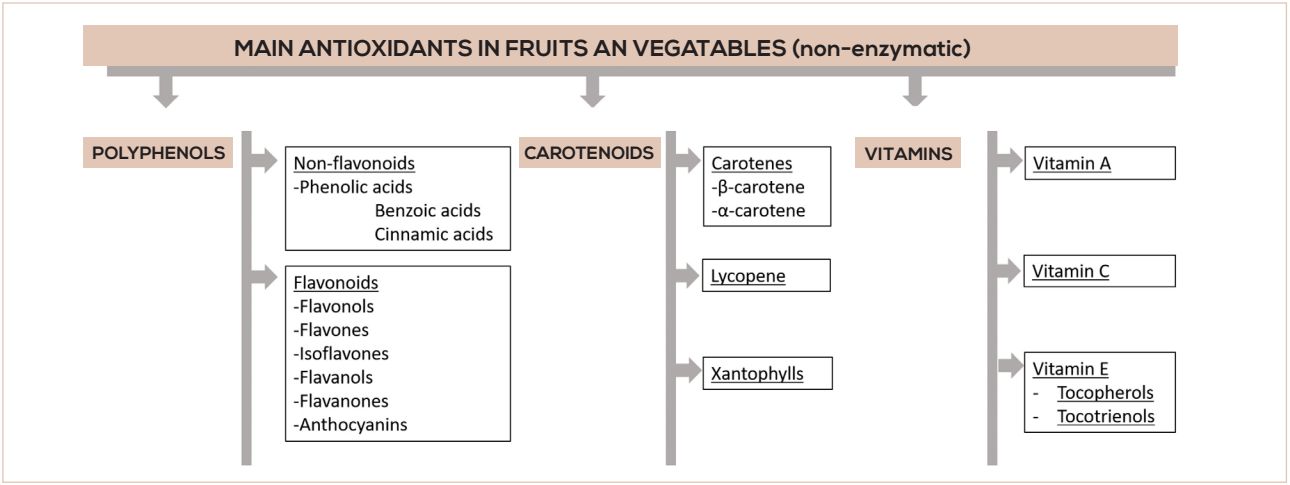
Furthermore, thanks to the various epidemiological studies conducted to date, it has been shown that vegetable consumption is positively associated with the prevention of several degenerative diseases for its bioactive compounds [49]. It has also been shown that people who consume fruit and vegetables as part of their routine meals are less affected by various chronic diseases [50]. Studies have also supported the long-term health impact of consuming these naturally-occurring diets [47,50]. Hence high and varied consumption of fruit and vegetables is directly associated with increased health benefits for reducing the risk of chronic diseases like cancer, cardiovascular disease, type II diabetes, arthritis and obesity, and even for slowing down ageing [45,50,51]. Fruit and vegetables have, thus, acquired the status of **"functional foods"** capable of promoting good health and preventing, or alleviating, disease [50], which are mainly attributed to the presence of antioxidants [38,46].

2.3. The main antioxidants in fruit and vegetables

Many nutritional studies focus on examining foods for their protective and disease-preventive potential by assessing antioxidants traits [50]. Likewise, consumers also prefer foods rich in antioxidants because they are aware of their potential to protect against free radical damage in not only food, but also in the human body [52].

Antioxidant compounds are, therefore, defined as the defence system against damage from reactive oxygen species (ROS) which normally occur during various physiological processes in the living organism [47], and to prevent uncontrolled cellular oxidation [38]. Antioxidants neutralise free radicals by donating one of their own electrons, and do not become free radicals themselves by donating electrons because they are stable in any chemical form [50]. ROS are partially reduced forms of oxygen, such as singlet oxygen (102), hydrogen peroxide (H_2O_2), superoxide (O^{2-}) or hydroxyl radical (OH^-), and are capable of causing detrimental modifications in proteins, lipids and nucleic acids by disrupting the normal metabolism of living organisms [38].

In general, antioxidants can be classified into two groups, synthetic and natural [53], along with those of natural origin that are preferred by consumers and come with more legislative approval than synthetic additives [52).



*** Figure 1:** The main antioxidants in fruit and vegetables.

The main ingredients in natural sources are polyphenolic compounds (but not the only ones), which are stated to have a significant antioxidant potential and are present in all plant parts [47,52]. The antioxidants obtained from vegetables are mostly phenolic, together with vitamins and minerals and polyphenols [47]. However, minerals like iron, zinc, selenium, copper and manganese act as cofactors for many antioxidant enzymes [47]. The main antioxidants present in fruit and vegetables are shown in Figure 1.

2.3.1 Polyphenols

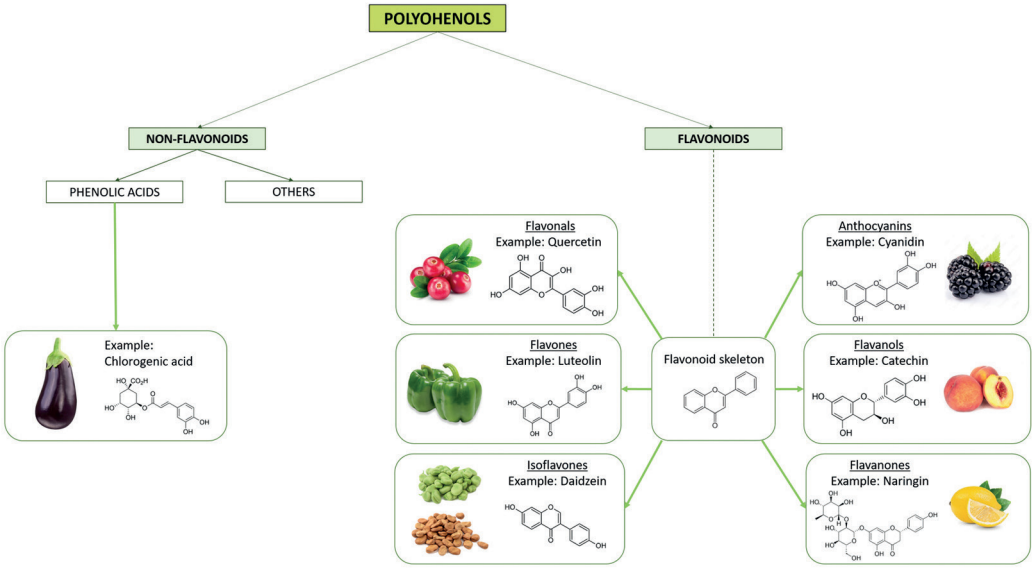
Polyphenols are the main secondary metabolites in plants and come in various structures, such as lignin, tannins, phenolic acids, flavonoids and numerous derivatives [49] (Figure 2). They form a group of various low- and high-molecular-weight compounds with antioxidant properties that prevent mainly lipid oxidation [49]. Most are conjugates of mono- and polysaccharides connected to one phenol ring group or more, or might also occur as functional derivatives, such as esters and methyl esters [47]. They are characterised by an aromatic or phenolic ring structure with varying degrees of hydroxylation [38,44]. Likewise, the structure of phenolic compounds is directly related to their antioxidant properties, with a higher degree of hydroxylation being indicative of higher antioxidant capacity [38]. The antioxidant activity of phenolic acids may be enhanced by other electron-donating groups associated with rings [38].

Plant metabolites are produced for their own growth and reproduction, with polyphenols being related to fruit pigmentation, UV protection, predator deterrence due to the bitter taste conferred by some polyphenols, and increased resistance to plant pathogenic fungi [38,44]. Luckily, they are also essential for human health given their ability to suppress oxidation processes [49].

Antioxidant and anti-inflammatory activities, along with other biological functions of polyphenols, are attributed to

their chemical structure. Aromatic structure and numerous hydroxyl groups make these compounds good electron and hydrogen atom donors by neutralising free radicals and other ROS [49]. As with other compounds, the health-promoting effects of polyphenols depend on their bioavailability [49]. In this context, several nutritional, clinical and epidemiological studies have shown that polyphenols support health and prevent various neurodegenerative diseases, including cancer and metabolic disorders, even if the concentration of these chemicals is very low in our organism [38]. Today the recommended daily intake of polyphenols is 1.177 mg for men and 1.192 mg for women [49].

As a large group of structurally diverse compounds, polyphenols can be classified in many ways. Based on their chemical structure, two main groups can be distinguished [54]: non-flavonoid polyphenols and flavonoids (Fig. 2).



*** Figure 2:** Polyphenols: classification, chemical structure and an example of a main food source (adapted from Goszcz, K. et al., 2015 or Frond 2018).

NON-FLAVONOID POLYPHENOLS

Phenolic acids, which are included in the non-flavonoid group, are hydroxylated derivatives of aromatic carboxylic acids that have a single phenolic ring and can be either non-carboxylic phenolics (C6, C6-C1, C6-C3) or phenolic acids (benzoic acid derivatives C6-C1 and cinnamic acid derivatives C6-C3) [49,54]. Phenolic acids are derivatives of benzoic and cinnamic acids. The most abundant benzoic acid derivatives are p-hydroxybenzoic, vanillic, syringic and gallic acids, while common cinnamic acid derivatives are p-coumaric, caffeic, ferulic and sinapic acids. Derivatives differ in the aromatic ring's degree of hydroxylation and methoxylation [38]. The main dietary sources of this type of compounds are onion, tea, kiwi, coffee, among others [55].

FLAVONOIDS

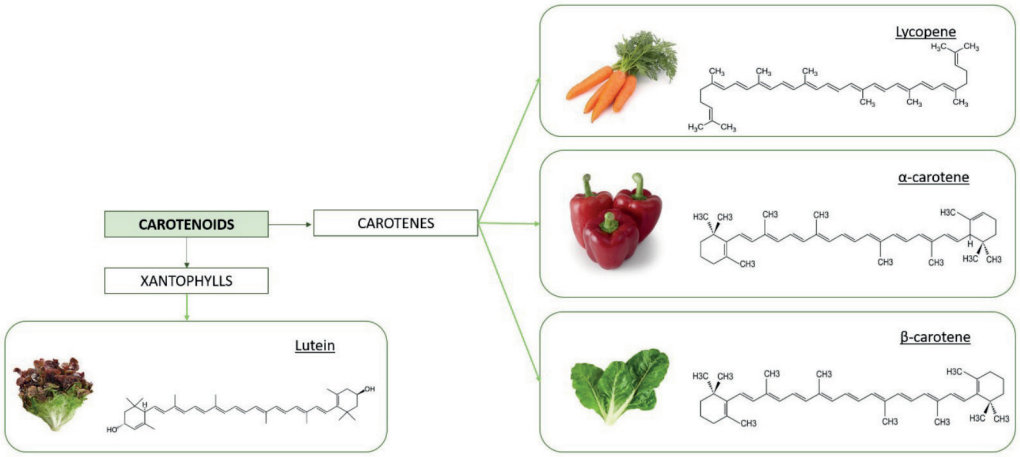
Flavonoids constitute the most important group in the polyphenols contained at high concentrations in vegetables [56]. In fact they are ubiquitous in vascular plants, often occur as glycosides [47,49,50], and more than 4,000 of these compounds have been identified [57]. Flavonoids are a large group of polyphenolic compounds with two aromatic rings associated by a carbon bridge of an oxygenated heterocycle, which has a C6-C3-C6 skeleton [38,49]. They are usually present in the form of glycosides, which are more soluble than the corresponding aglycones, and are compartmentalised in vacuoles. There are different subclasses of flavonoids: flavones and flavonols, flavanones and flavanols, isoflavones, proanthocyanidins and anthocyanidins (Ant) [38].

Interest in these compounds was initially shown in their bitterness, astringency, colour and odour. However, they were later recognised for their nutritional value and associated potential [49]. It is now known that dietary flavonoids possess antiviral, anti-inflammatory, antihistamine and antioxidant properties. In this context, they inhibit lipid peroxidation, scavenge free radicals, chelate iron and copper ions, and modulate cell-signalling pathways [44]. Many of these flavonoids have even been found to be more potent antioxidants than vitamins C, E and β-carotene [57]. Many of them, such as Ant, chalcones and flavones, are plant pigments that determine the colour of fruit and vegetables [58], and are mainly found in grapes and cranberries (GOszcz, 2015). Other types of flavonoids, such as catechin, quercetin, dihydroquercetin and rutin that appear mainly in onion, leaks, broccoli, cherries, peaches or cocoa (Goszcz, 2015), are known for their antioxidant properties [56]. Quercetin, which is part of a subclass of flavonoids called flavonols, is the main antioxidant component of vegetables [56]. Apart from Ant being related to colouring, they are also considered to be potent antioxidants, but their bioavailability is lower than that of other flavonoids [47].

2.3.2 Carotenoids

Carotenoids are another major class of antioxidant phytochemicals in fruit and vegetables after polyphenols [47]. Plant carotenoids (β-carotenes, α-carotenes, xanthophylls, lycopene) are orange, yellow and red fat-soluble pigments [38,44] (Figure 3). They are terpenoids that consist of eight isoprene (2-methyl-1,3-butadiene) units that derive from isopentenyl diphosphate. Those with an unsubstituted β-ring with an 11-carbon polyene chain possess pro-vitamin A activity, such as β-carotene, α-carotene and cryptoxanthin [38], and are essential for human diet [44]. There are about 600 known carotenoids in nature, most of which take the general C40H56On chemical structure, where n is the number of oxygen molecules that can vary from 0 to 6 [59]. Carotenoids are further subdivided into two classes: C- and H-containing carotenoids (e.g. β-carotene, α-carotene, lycopene) and oxygenated derivatives known as xanthophylls (lutein, violaxanthin, zeaxanthin) [38].

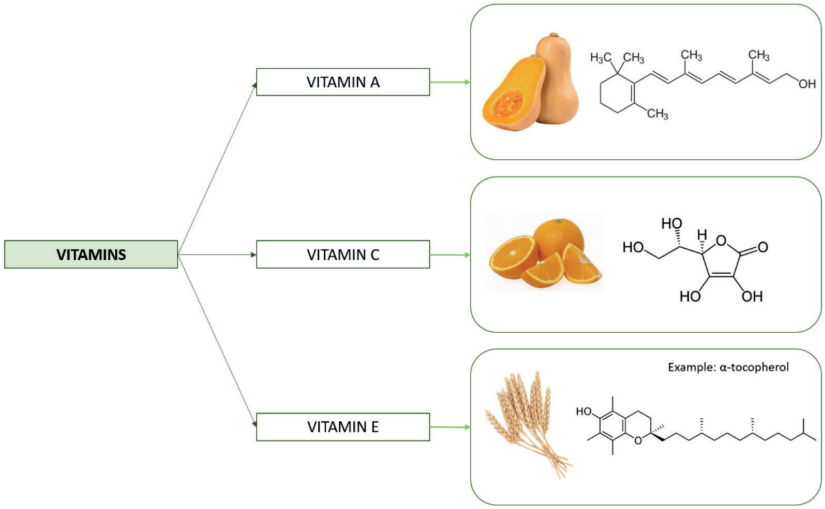
In plants, carotenoids protect photosynthetic tissues against photooxidative damage [38]. Furthermore they are precursors of the phytohormone abscisic acid, which modulates development and stress processes [44]. In the human health context, the presence of conjugated double bonds in carotenoids is the basis for their antioxidant potential activity and ability to prevent some pathologies [38]. Indeed a positive correlation has been observed between eating carotenoid-rich foods and being at lower risk of developing certain cancer types [44,50], oxidative stress, and degenerative [44]or chronic diseases [50].



* **Figure 3:** Carotenoids: classification, chemical structure and an example of a main source.

2.3.3 Vitamins

The main antioxidant vitamins are **vitamin A** (retinol), **vitamin C** and **vitamin E**. They do not share common functions or a structure, and are usually classified as fat-soluble (vitamins A and E) or water-soluble (viatmin C) [38].



*** Figure 4:** Main antioxidant vitamins: classification, chemical structure and an example of a main food source.

Vitamins in minimal amounts are necessary for normal development, and cannot be synthesised in sufficient quantities by the body, but must be obtained from diet. Fruit and vegetables are a vital source of vitamins, but concentrations vary according to species, cultivars, environmental conditions and cultural practices [38]. Collectively, vitamin C, vitamin E and β-carotene (precursor of vitamin A) are known as antioxidant vitamins par excellence [50] (Figure 4), which also contribute to good health by acting as cofactors for certain antioxidant enzymes or participating in oxidation-reduction reactions [56]. In this context, vitamin C (ascorbic acid) has been classified as being key for human health, which is why higher daily intakes are recommended [60].

VITAMIN A

The term vitamin A generically refers to the biologically active compounds of retinol all-trans (R-OH), which also include retinaldehyde (retinal) (R-CHO), several retinyl esters (the dominant form in foods) (R-OO), retinoic acids (R-OOH), among other active metabolic intermediates of vitamin A [59]. Furthermore, natural vitamin A compounds are considered a subset of a much larger "retinoids" family, and share a common, monocyclic and double-bonded chemical structure with several functional groups [59]. Carotenoids, which are found mainly in plants, mostly β-carotene (carrots, spinach, sweet potatoes) and α-carotene (carrots, pumpkin, red and yellow peppers) [44], provide the precursor form of vitamin A [59].

Vitamin A regulates many key biological processes in humans because it is involved in morphogenesis, growth, vision, bone development and reproduction [38,61], and is also crucial in cell division, differentiation and proliferation [59]. Vitamin A is also a recognised potent regulator of gene expression [61]. The daily adult vitamin A requirement is estimated at 5,000 international units (1 IU is 50.3 µg of retinol or 0.6 µg of **β**-carotene), and fruit and vegetables provide up to 30% of this amount [38].

VITAMIN C

Ascorbic acid (vitamin C, AsCH2) is a water-soluble ketolactone with two ionisable hydroxyl groups [50,62]. The ascorbate monoanion (AscH), is the dominant form of our organism at a physiological pH. It is an excellent reducing agent that forms ascorbate radicals (Asc--) and dehydroascorbic acid (DHA) upon oxidation [62]. Ascorbic acid (AsA) and its first oxidation product, DHA, are considered vitamin C. AsA is a carbohydrate-derived compound with antioxidant and acidic properties due to a 2,3-endiol moiety (Vincente, 2014). In addition to its vitamin functions, the role of AsA in disease prevention is associated with this ability to neutralise ROS [38]. As vitamin C is a potent biological antioxidant, its major health benefits are generally recognised. Indeed it has been linked with the prevention of degenerative diseases, such as cataracts, certain cancers and cardiovascular disorders [44], and its antioxidant activity is due to the ease with which it loses electrons, which makes it a potent and effective reducing agent in biological systems [50]. Vitamin C is also involved in the synthesis of neurotransmitters, steroid hormones and collagen, the conversion of cholesterol into bile acids, and iron and calcium absorption. It helps to heal wounds and burns, to prevent blood clotting and bruising, and to strengthen capillary walls [44].

Fruit, vegetables and juices are the main dietary sources of vitamin C [47], which comes at high concentrations, particularly in citrus fruit, cantaloupe, mango, strawberries, and peppers (64). In fact these products are estimated to account for 90% of the total dietary supply of vitamin C [38]. It is also present in oranges, tangerines, grapefruit or kiwi. The recommended dietary allowance for vitamin C is 75 mg and 90 mg per day for young men and women, respectively [38]. It is also recommended that the fruit and vegetables containing vitamin C are eaten in small separate doses rather than simultaneously taking a large dose because vitamin C is less well absorbed when administered in large amounts [47].

VITAMIN E

Vitamin E is the most abundant lipid-soluble antioxidant [50], and includes both tocopherols and tocotrienols. These compounds exist in eight related molecular forms: four tocopherols and four tocotrienols [38]. The structure of both groups consists of two primary parts: a complex chromanol ring and a long side chain. The chromanol ring can present different substitution patterns of methyl groups at positions 5, 7 and 8, designated as **α**-, **β**-, **δ**- and **γ**-. However, they differ in side chain saturation because tocopherols have a saturated chain and tocotrienols an unsaturated one with three double bonds at carbons 3, 7 and 11 [63,64]. Each form has vitamin E activity, but **α**-tocopherol is the most active [38,64]. In fact many studies have shown a direct association between vitamin E intake and a reduced risk of several chronic diseases. **α**-tocopherol may act directly by inhibiting the oxidation of low-density lipoproteins (LDL) and indirectly through specific molecular functions related to the modulation of the activity of the enzymes and molecules involved in cell signalling and gene expression [50,65]. Vitamin E is also present in foods like rice bran, cereals, palm and green vegetables (Mizacawa, 2011). The average recommended vitamin E intake for both sexes is 15 mg of **α**-tocopherol per day, based on the estimated

3. VALENCIAN CROPS WITH AN IMPORTANT STOCK OF TRADITIONAL VARIETIES

3.1. Pepper

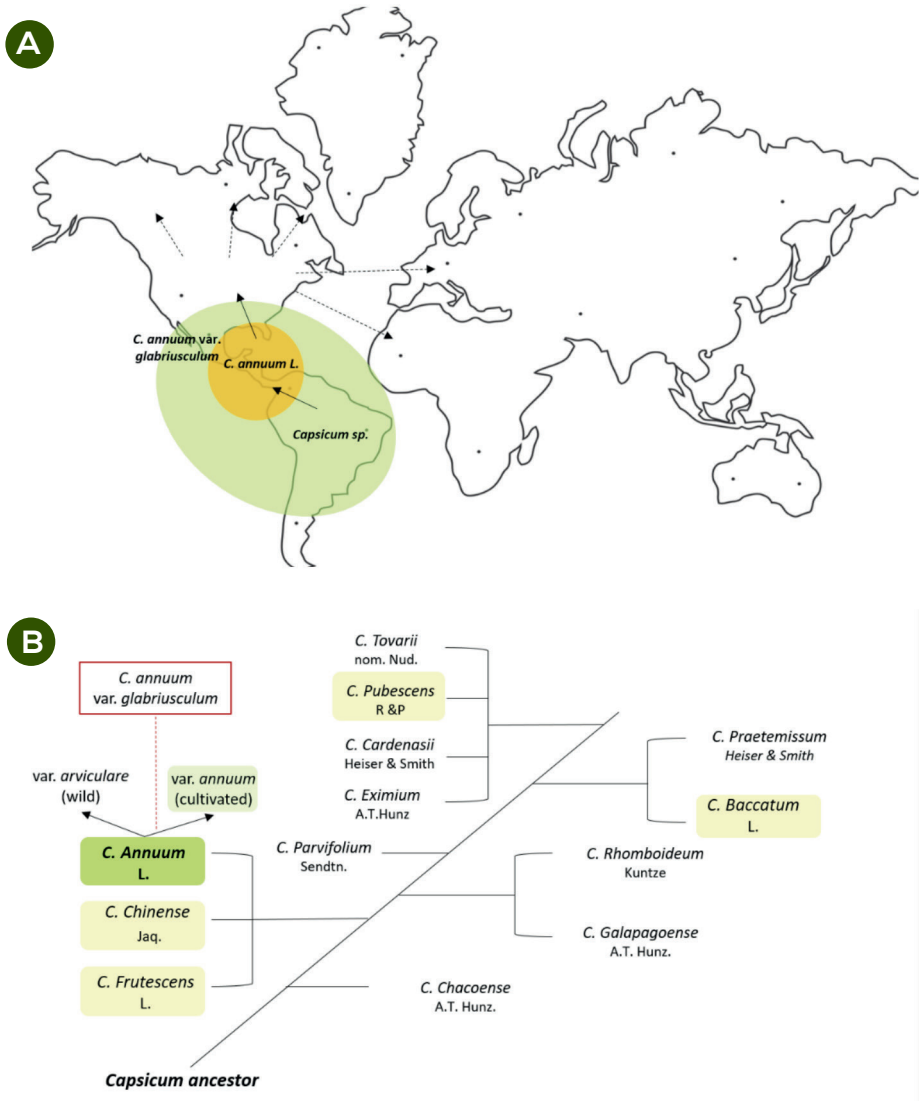
3.1.1 Taxonomy

According to (FAO, 2008), the pepper varieties used in this doctoral thesis come with the following taxonomic classification:

Kingdom----------Plantae
Division---------- --Magnoliophyta
Class ----------Magmoliopsida
Order----------Solanales
Family----------Solanaceae
Genus----------Capsicum
Species----------***Capsicum annuum L.***
Common name----------Pepper

3.1.2 Origin

The genus ***Capsicum sp***. is believed to be native to the tropics and subtropics of America [66,67]. Data suggest that it was the first domesticated and cultivated spice crop. The earliest record of pepper use comes from archaeological excavations in the Tehuacán Valley, Mexico (8500 BC) [68]. The starch that derives from peppers has been found preserved at archaeological sites located in the region from the Bahamas to South America (6000 BP) [66,68,69]. More specifically, Hunziker [70] proposes four distribution centres of ***Capsicum***: 1) from southern USA and Mexico to western South America; 2) north-eastern Brazil and coastal Venezuela; 3) the eastern coast of Brazil; 4) central Bolivia and Paraguay to northern and central Argentina [67]. In each area of origins, one species was, or more species were, domesticated and then dispersed to different areas, where they continued to be selected to give rise to distinct morphological pepper types, especially in fruit traits [68]. Today they are distributed worldwide and cultivated for use as spices, vegetables and ornamentals, especially in temperate tropical areas [68](Figure 5A).



*** Figure 5:** A) Distribution of the genus ***Capsicum*** and the possible origin of ***C. annuum*** L. Arrows indicate the proposed migration of wild ***Capsicum*** species through the domestication of C. ***annuum*** L. The currentl C. ***annuum*** L. is cultivated worldwide and its specific range is not shown on the map. B) Diagram showing the possible evolutionary relations between ***Capsicum*** species based on phenotype and karyotype studies.

As Orarat and Taylor [67] explain, to date, 31 species have been identified, of which **five** are **domesticated:** ***C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens*** [67,68]. All ***Capsicum*** species share a common ancestor, which is a diploid with a basic chromosome number of 12 (2n = 24). C. chacoense is the species that would have differentiated in the earliest stage of the evolutionary line, followed by the C. ***annuum complex comprising*** C. ***annuum, C. chinense and C. frutescens*** [67]. The primitive group, represented by white-flowered species, ***comprises C. galapagoense, C. rhomboideum and C. parvifolium.*** The recent species group starts with C. ***baccatum and C. praetermissum***, and three more advanced groups, followed by the group of purple-flowered species (C. ***eximium, C. cardenasii, C. pubescens and C. tovarii***) [67] (Figure 5B).

C. annuum is the most widely cultivated species [69]. It is believed to have originated in the central part of the continent from Colombia to the southern United States and the Caribbean Islands because this is the distribution of C. ***annuum var. glabriusculum*** (Dun.), a wild species considered to be the most likely progenitor of the cultivated varieties corresponding to taxon C. ***annuum var. annuum*** [71] (Figure 5).

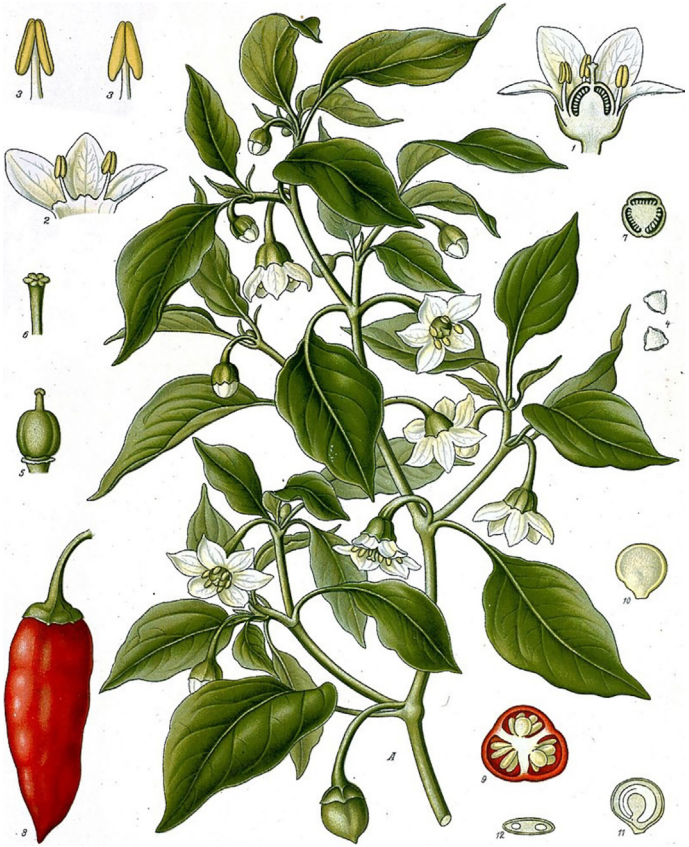
C. annuum varieties are distinguished by fruit characteristics, such as taste (sweet or spicy), size, shape and colour [71]. The sweet pepper varieties are specifically intended for fresh consumption or industry. Accordingly, most pepper production in Spain centres on this type of varieties because 70.8% of pepper is cultivated in greenhouses, and 90.56% of cultivated peppers are consumed fresh [72]. It is also possible to divide the sweet pepper varieties into four subgroups according mainly to fruit morphology: 1) California type: short fruit (7–10 cm), wide (6–9 cm) with three or four loculi, a calyx and thick flesh (3–7 mm); 2) Lamuyo type: large fruit (13–15 cm long, 8–10 cm wide) with three or four loculi; 3) Italian type: fruit 16–17 cm long and 4–5 cm at the base, elongated, narrow, pointed and thin-fleshed; 4) Marconi type: pendulous fruit, 13–18 cm long and 8 cm wide with three or four well-marked loculi, sweet flesh, and eaten both green and red [73].

3.1.3 Botany

C. annuum L. varieties present very high **morphological diversity**. For this reason, internationally accepted official descriptor sheets have been generated to encompass all morphological characters and to serve as a criterion to classify genetic resources [71](Figure 6). Peppers are herbaceous or semiwoody perennial plants with an annual cycle depending on the climate conditions of the area where they grow [71,73–76].

The root system is pivotal and deep with numerous adventitious roots that can horizontally reach a length of 50–100 cm [71,73,74].

The plant is composed of a lignified, erect and branched main stem of varying height (usually from 0.5 m outdoors to 2 m in the hybrids grown in greenhouses), with 2–20 internodes that end in a flower. Its last node branches into two or three secondary stems with each secondary stem to subsequently show the same pseudo-dichotomous growth pattern [71,73].



*** Figure 6:** Botanical illustration of a ***Capsicum annuum L.*** plant (adapted from Köhler [77]).

Leaves are entire and of an oval lanceolate shape with regular margins and an acuminate apex [73]. The upper side is glabrous and colour depends on variety (usually bright, intense and dark green). The main vein starts at the leaf base as an extension of the petiole, as do secondary veins, which are pronounced and almost reach the leaf margin. Insertion of leaves into the stem is alternate [74].

Flowers appear individually at each stem node (rarely grouped in 2 or 3), with an insertion into the axils of leaves. They are small and consist of a generally white corolla with five petals welded together, a calyx with five green sepals welded together and five stamens, with elongated anthers and longitudinal dehiscence [73]. Pepper plants are self-pollinated [74], although 10%-90% of allogamy occurs in outdoor crops due to insect-mediated cross-pollination.

Thus some authors consider pepper to be a facultative cross-pollinator [71].

Fruit are hollow berries of variable taste (sweet or spicy), size (from a few grams to more than 500 g), shape (cuboid, conical, pyramidal, elongated, round, etc.) and colour (green, red, yellow, orange, violet or white) depending on variety and ripening stage [71,73]. They can weigh from a few grams to more than 500 g [74]. Seeds are embedded in the placenta. They are pale yellow and reniform-shaped when ripe [71,76].

3.1.4 Crop requirements

According to Deker [73], the cultivation of peppers requires warm temperatures, and the optimum temperature range is 21-31ºC. Peppers require considerable light, especially in the initial growth stage, as well as environmental relative humidity (RH) over 70%. They also require deep well-drained soil with 3-4% organic matter content [73]. A soil pH range of 5.5- 7.0 is necessary to grow them [73,74].

3.1.5 Nutritional quality

Peppers are consumed fresh, dried or processed as various food types around the world [69]. Hence they are highly valued for their high content of fibre, carbohydrates, minerals (mainly iron, magnesium, phosphorus and calcium [78]) and vitamins [66], especially vitamin C and other antioxidant compounds like carotenes (responsible for ripe fruit colour), phenols, capsaicinoids, xanthophylls and flavonoids [79]. Factors like ripening, environmental conditions or storage determine the content of these biocompounds [78].

It is considered one of the most important exported fruit worldwide [80]. The micronutrients provided by peppers have boosted the use of this crop in traditional medicine to prevent degenerative diseases, intestinal disorders, dysentery [5], different mental health-related problems [6], among other ailments [79].

3.1.6 Economic importance

The largest fresh pepper producer is the Asian continent (65.1% of world production), followed by America (13.5%) and Europe (11.1%). According to the FAO, in 2020 more than 36 billion tons of fresh pepper were produced, with more than 2 million hectares (ha) of land used to grow them [81]. Data indicate a 17.9% rise in production and a 9.56% increase in cultivated areas over the last 10 years. Of the principal production countries, Spain ranks fifth for covering 4.1% of total fresh production [81]. The Valencian Community covers 5% of Spanish production for cultivating 69,808 tons of pepper on 839 ha of land in 2020 [72].

When analysing dry pepper production, Asia still leads with 72.5% of total production, followed by Africa (19.9% of total production). For such cultivation, Spain is not considered one of the main producing countries [81].

3.2. Eggplant

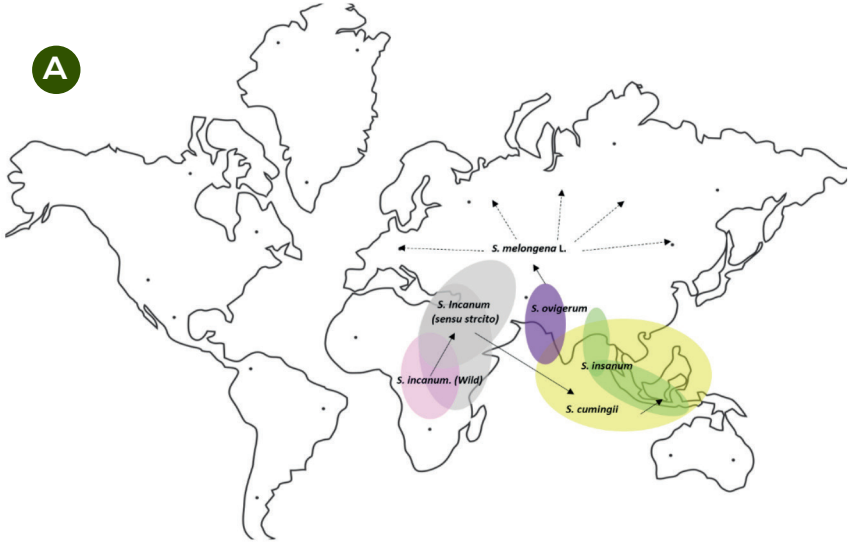
3.2.1 Taxonomy

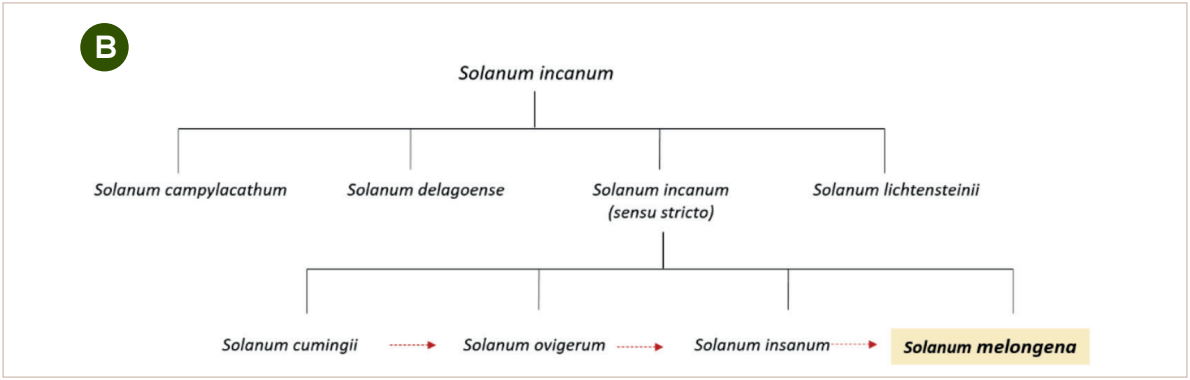
According to (FAO, 2008), the cultivated eggplants used in this doctoral thesis come with the following taxonomic classification: :

Kingdom ---------- Plantae
Division ---------- Magnoliophyta
Class ---------- Magmoliopsida
Order ---------- Solanales
Family ---------- Solanaceae
Genus ---------- *Solanum*
Species ---------- *Solanum melongena L.*
Common name ---------- Eggplant, aubergine or brinjal

3.2.2 Origin

Eggplant, has been cultivated for centuries [82]. Records state that it has been used as food for over 2,000 years in China and for 4,500 years in India, according to traces of ***Solanum*** on Harappan cooking vessels [83]. By the year 1,200 it was already cultivated in Egypt, from where it was incorporated into the Iberian Peninsula and Turkey in the Middle Ages [84]. According to Barraza [85], it then spread throughout the Mediterranean Region and the rest of Europe. Today, it is cultivated worldwide [82] (Figure 7A).





*** Figure 7:** A) Distribution of ***Solanum*** species. Arrows indicate the proposed migration of the wild species of ***S. incanum*** groups through the domestication of ***S. melongena***. The ***S. melongena*** L. cultivated today is grown worldwide and its specific range is not shown on the map. B) Diagram showing the possible evolutionary relations between ***Solanum*** species based on phenotype and karyotype studies.

According to the studies by Lester and Hasan [86], the ancestral forms of ***S. melongena*** originated in the African tropics, where it is possible to find many wild relatives of aubergine today. In particular, ***S. incanum*** L., a complex taxon that groups several wild species found in East African and Middle East Asian countries, has been shown to be the closest and, thus, the most likely progenitor to ***S. melongena*** [83,87]. The cultivars included in ***S. incanum*** would have migrated to Asian eastern countries either spontaneously or as a result of human migrations [86,88], which would have enabled its domestication in the Indo-Burmese region, where several documents dating back to 300 BC suggest that aubergine was very popular as food and a source of medicine [88].

The ***S. incanum*** group comprises four species named ***S. campylacathum*** (distributed in the East African tropics, ***S. delagoense*** (located in southeast Africa), ***S. incanum sensu stricto*** (distributed in northeast Africa and the Middle East) and ***S. lichtensteinii*** (located in southeast Africa) [82]. Furthermore, ***S. incanum sensu*** stricto progressively differentiates into closely related species: in southeast Asia ***S. cumingii and S. melongena*** (distributed from southern and eastern India to southern China, the Philippines and Indonesia). When ***S. cumingii*** was domesticated, it gave rise to the early aubergine cultivar known as S. ovigerum, a cultivar with small, round or oblong, white, green or violet fruit. In turn, ***S. ovigerum*** progressively evolved into advanced cultivars with large fruit and is currently known as S. insanum (widespread in India), which is probably a form of ***S. ovigerum*** that reverted into the wild state to develop marked thorniness, a low straggling growth habit and a short life cycle [88]. Lester and Hasan [86] group all these taxa under the umbrella of ***S. melongena***, structured as ***S. insanum, S. cumingii and S. ovigerum***, and advanced cultivars (the ***S. melongena*** cultivated today) [88] (Figure 7B).

3.2.3 Botany

The domestication, natural interbreeding, human selection and hybridisation of this crop have resulted in very high genetic diversity for eggplant cultivars, although the most widely cultivated species is ***Solanum melongena*** [89]. Unlike primitive aubergines, which are tall plants with large spiny leaves that flower in clusters and grow small, green, bitter-tasting fruit with thick skin and tough flesh [89], ***S. melongena*** differs from its wild predecessors mainly in fruit colour and shape (Figure 8). Cultivated aunergine fruit range in colour from dark purple to black, with some green and white varieties, are much larger than the wild type and more variable in shape [90]. Aubergine cultivars can also be differentiated by vegetative parts, chemical fruit composition, earliness of fruiting, yield and environmental requirements [89].

Eggplant is a diploid species with a basic chromosome number of 12 (2n = 24) and a genome size of approximately 956 Mbp [67,90]. Despite its bushy appearance, it is an herbaceous plant due to its lignified stems [84], and is annual, but can resprout in a second year when it is properly cared for and pruned [91]. It grows a pivotal, vigorous and highly capillary root system. Hair constitutes up to 70% of the root system [84,91].

Anthocyanin content, spines and the hairiness of vegetative parts vary quantitatively between cultivars [88].

Leaves are large and entire (15 cm to 25 cm) [91], and are alternately attached to the plant stem [91]. The upper side has a long petiole and a distinct rib with spines, while the underside is covered with greyish hair [92].

Hurtado [93] stated that the eggplant is known for having pentamerous **flowers** with purplish petals and constant sepals [84] that can lead to botrytis attacks (***Botrytis cinerea***) when RH is high because petals are trapped between the calyx and fruit [91]. Flowers with six to nine petals can be found, and are usually associated with the globular and rounded fruit types [83]. The **ovary** is superior and bilocular [84], while stamens have highly developed yellow anthers below the stigma, which make direct fertilisation difficult [91]. Eggplant is generally considered an autogamous species, although the allogamy rate can reach 70% or higher in open fields and under warm conditions due to the mediation of pollinators [88].

*** Figure 8:** Botanical illustration of ***Solanum melongena L.*** (adapted from Pignatti [94]).

Fruit are berries characterised by their variable colour (light to dark purple, almost black, green or white), length (4-45 cm), thickness (2-35 cm), shape (diverse) and weight (15-1,500 g) [89]. Colour is determined mainly by the absence/presence or distribution of chlorophylls and Ant in the epidermis [88], which allow colour oscillations between the different genotypes and ripening stages of fruit [84].

3.2.4 Crop requirements

Eggplant is high-yielding and well-adapted to hot and wet environments [90], and can tolerate temperatures up

to 40-45ºC. For its correct development and quality however, it requires optimal temperatures of 21-29ºC, with an average maximum temperature of 35ºC and an average minimum of 18ºC [91]. Aubergine does not tolerate excessively dry climates and very high temperatures because a high transpiration level causes plants to wilt [92]. Thus the optimum air RH air lies between 50% and 70% [95]. In addition, it is very light-demanding (10-12 hours of light per day). The most suitable soils for eggplan cultivation are sandy-clay soils rich in nutritional principles with pH between 6 and 8.5. Acid soils imply growth and production problems [91,95].

3.2.5 Nutritional quality

In nutritional value terms, eggplants have a very low calorie value. They are considered one of the healthiest vegetables given their high content in vitamins, minerals and bioactive compounds for human health [96]. Its fresh weight is composed of 92.7% moisture, 1.4% protein, 1.3% fibre, 0.3% fat and 0.3% minerals. The remaining 4% is composed of several carbohydrates and vitamins (A and C) [87].

Aubergine ranks among the top 10 vegetables in terms of oxygen radical scavenging capacity [97]. Plazas [96] associates bioactive eggplant properties with its high phenolic contents, which are mainly phenolic acids in flesh and Ant in fruit skin [97].

This product has been used in traditional medicine for asthma, bronchitis, cholera and dysuria. Fruit and leaves are also known to be beneficial for lowering blood cholesterol and they possess antimutagenic properties [87,96].

3.2.6 Economic importance

Eggplant is a widespread crop worldwide, with the largest producer being the Asian continent (94% of world production), while Europe ranks third (1.7% of world production). According to the FAO, in 2020 more than 56 billion tons of fruit were produced, with over 1.8 million ha of land used to grow it [81]. Data indicate a 25.6% increase in production and 7.5% growth in cultivated areas in the last 10 years.

According to the data from the latest Yearbook of Agrifood Statistics [72], 3,701 ha of land are reserved for aubergine cultivation in Spain. In 2020, the total production in Spain was 282,200 tons, and 179,826 tons in 2007 (36.3% higher eegplant production). However, the harvested area is practically the same [81]. In Spanish provinces, the Valencian Community ranks sixth with 4.5% of the total Spanish production [72]. According to export rates, in 2007 Spain occupied first place in the market with 91,834 tons, which represents 51% of the country's total production [72].

3.3. Lettuce

3.3.1 Taxonomy

According to (FAO, 2008), the pepper varieties used in this doctoral thesis come with the following taxonomic classification:

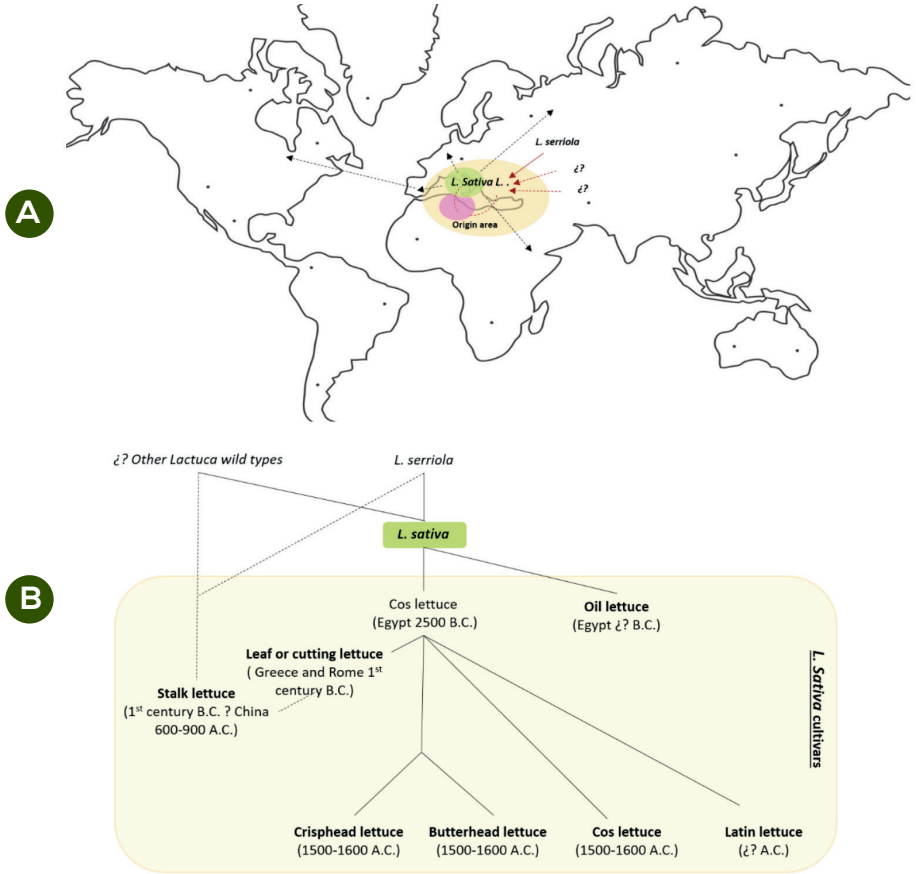
Kingdom ---------- Plantae
Division ---------- -- Magnoliophyta
Class ---------- Magmoliopsida
Order ---------- Asterales
Family ---------- Asteraceae
Genus ---------- *Lactuca*
Species ---------- *Lactuca sativa L*.
Common name ---------- Lettuce

3.3.2 Origin

Lettuce is native of warm regions in Europe, Asia and North America.The lettuce cultivated today is considered to have originated in the Mediterranean Region [98,99], and the Middle Eastern area (Egypt and Iran) is considered the heart of the origin of lettuce [99]. In particular, there is evidence from 680 BC of using lettuce in Egypt to produce seed oil [98], together with many illustrations on Egyptian tombs displaying very similar lettuce-like plants to those found today in the same region. Furthermore, the existence of early forms in the Middle East supports the notion that lettuce probably originated in the eastern Mediterranean Basin [100]. From Egypt, cultivated lettuce spread to Greece and Rome and throughout the Mediterranean Region [100]. This plant was introduced to America by Christopher Columbus in the late 15th century. Today lettuce consumption is widespread worldwide thanks to its high nutritional value and medicinal importance [98](Figure 9A).

It is still unclear which species were involved in the evolution that has led to today's lettuce. The species ***L. sativa*** is characterised by the high genetic diversity that results from its polyphyletic origin and a complex domestication process [99]. However, the species ***Lactuca serriola*** has been proposed as the, or the only, direct ancestor [101]. The chromosomes of ***L. sativa*** and ***L. serriola*** are similar morphologically, and can easily interbreed. Most likely the changes in ***L. serriola*** caused by mutations led to the emergence of forms that were favoured by humans, particularly forms without prickles on stems and leaves, and with large seeds [101]. Similarly, Lindqvist [102] claims that current lettuce cultivars are the result of human selection in a large gene pool of ***L. serriola***, with the simultaneous introgression of genes from other ***Lactuca species*** or, alternatively, as an independently selected species. Cytogenetic studies have shown that the primary gene pool of ***L. sativa*** is represented by its numerous cultivars, primitive landraces and wild species without crossing barriers. Among them,

cosmopolitan species ***L. serriola***, together with ***L. aculeata, L. scarioloides, L. azerbaijanica, L. Georgica, L. altaica*** (from Asia), ***L. dregeana*** (from South Africa) [103], ***Lactuca saligna*** and ***L. virosa*** (from Europe), feature among other wild species that can be crossed with ***L. sativa*** with varying degrees of compatibility [99]. Of the mentioned species, only four can be crossed with each other by conventional hybridisation methods and, thus, form the most important breeding groups: ***L. sativa***, ***L. serriola***, ***L. saligna*** and ***L. virosa*** [101]. The actual edible genotypes are arranged into seven groups of lettuce cultivars described as morphotypes: ***Butterhead, Crisphead, Romaine, Leaf, Stem, Latin*** and ***oil lettuce***. The most recent reviews of the taxonomic and phenotypical analyses of lettuce cultivars are those shown in Figure 9B.



*** Figure 9:** A) Area of origin of ***Lactuca sativa L.*** Arrows indicate the proposed species migration, together with the most likely ancestor ***L. serriola***. Currently, ***L. sativa*** L. is cultivated worldwide and its specific range is not shown on the map. B) Diagram showing the possible evolutionary relations between the ***Lactuca species*** and the ***Lactuca sativa L.*** varieties (morphotypes) based on phenotype and karyotype studies.

3.3.3 Botany

Lettuce is an herbaceous, annual and autogamous plant (Figure 10). The lettuce plant goes through four development phases: 1) **Seedling:** with the appearance of the radicle, emergence of cotyledons and deep root growth; 2) **Rosette period:** when new leaves appear with a lower leaf length-width ratio and final rosette formation made up of 12-14 leaves [104]; 3) **Head formation:** when leaves are even wider and curved along the axis of the nerve, which allows new leaves to be wrapped by previously formed leaves; 4) **Flowering**: when stem elongation signals the end of the vegetative phase and the reproductive phase begins [101]. Then heads lose quality, become elongated and finally promote the emission of inflorescences [104].

The root system of lettuce is characterised by the development of a deep (no more than 25 cm long) and short tap root with largely horizontal lateral roots that become denser near the soil surface to absorb water and nutrients [54,101].

Leaves are practically sessile and spirally arranged in a dense rosette structure around the stem. There is considerable diversity in leaf colour, shape, surface, margin and texture in the different types and forms of lettuce cultivars. Leaf margins may be entire, lobed, incised, toothed or wavy. The leaf surface may be smooth, savoy or wrinkled. The colour of lettuce leaves ranges from yellow to dark green to purple [54], with varying degrees of glossiness. The Ant concentration on the leaf surface can vary and range from none or only the margin of the leaflet to the entire leaf [101].

A single small **stem** normally forms that does not usually branch, at least not until flowering [54]. In the flowering phase, the stem elongates (1 to 1.5 m) and branches form in the distal area. Each one supports an inflorescence, formed by a dense corymbose panicle composed of yellow flower heads arranged in clusters. The number of florets usually ranges from 12 to 20, where each flower is provided with a feathery pappus [54], and consists of five stamens and a monocular ovary of a divided stigma, with an ovule [104], which produces a single-seeded achene. The seed is 4-5 mm long and its colour is generally creamy white, but can also be brown and chestnut. Its maturation occurs between 12-15 days after flowering [104].

*** Figure 10:** Botanical illustration of ***Lactuca sativa L.*** (adapted from Krauss, [105]).

3.3.4 Crop requirements

Lettuce grows well in cool temperate climates with monthly temperatures ranging between 7ºC and 24ºC [104], with the optimum temperature range from 18ºC to 20ºC. At high temperatures, plant growth is impeded and early flowering is induced (an undesirable phenomenon called "gleaning"). Nevertheless, this crop better withstands high temperatures than low temperatures [54]. The lettuce root system is very small compared to the aerial part and is, thus, very sensitive to lack of humidity and hardly resists drought, even very short periods. The suitable air RH for lettuce is 60-80% [104]. Lettuce is a short-day vegetable, and its development is greater when the photoperiod lasts less than 14 hours of sun [104]. Although lettuce grows well in different soils, it is preferably grown in fresh loamy soils that do not retain excess moisture, and with high organic matter content and a pH not above 7.4 [54]. The most favourable pH range for lettuce is 5.2 to 6.7 [104]).

3.3.5 Nutritional quality

In nutritional value terms, the contribution of the lettuce plant to diet is minimal because 95% of its fresh weight is water. However, as it is generally eaten raw in salads, a higher percentage of nutrients is retained in its intake compared to other cooked crops, such as potatoes [106,107]. Today, lettuce is a very popular vegetable. When its total consumption volume is considered, it ranks fourth behind tomato, citrus fruit and potato in terms of its overall contribution to nutrition [106]. In addition to water, the chemical composition of lettuce is 1.4 g of protein, 2.2 g of carbohydrates, 1.1 g of dietary fibre, 0.2 g of fat and 1.2 g of ash in 100 g of lettuce leaves [98]. Lettuce is also rich in vitamins, especially vitamins A, C and K, niacin and folate. It is also rich in many minerals like calcium, iron, phosphorus, sodium and potassium [54,98]. Furthermore, the healing properties of lettuce are partly determined by its content of antioxidant compounds, mainly vitamin C and polyphenols [108]. Recent studies in rats and humans have demonstrated the health-promoting effects of lettuce to prevent cardiovascular diseases [108].

Lettuces have also been used in traditional medicine for many decades to treat inflammation, pain, stomach problems, bronchitis and urinary tract infections [101]. There is even scientific evidence that the biological activities of lettuce include antimicrobial, antioxidant and neuroprotective properties [98].

3.3.6 Economic importance

Lettuce is considered the most important leafy vegetable crop. It is almost exclusively used as a fresh vegetable in salads, but some forms are also cooked [99]. It is a widespread crop worldwide, with the largest producer being the Asian continent (60.5% of world production), followed by America (22.1%) and Europe (15%). According to the FAO, in 2020 almost 30 billion tons of lettuce were produced, with more than 1.2 million hectares of land used to grow this crop [81]. Data indicate 11.9% growth in production and a 7.1% increase in cultivated areas in the last 10 years. The principal production countries include China, the United States of America, followed by Spain and Italy, with 51.76%, 15.91%, 4% and 3.5% of total production, respectively [81]. In 2020, the total production in Spain was 961,938 on 34,005 ha [72]. The Valencian Community grows 7.1% of all Spanish production and produced 68,450 tons of lettuce on 2,502 ha of land in 2020 [72].

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CHAPTER 02

THESIS OBJECTIVES

The present study forms part of a larger project conducted in the Horticulture Department of the Valencian Institute of Agriculture Research (IVIA) and the Conservation and Improvement of Valencian Agrobiodiversity (COMAV), whose main objective is to make an inventory of Valencian plant genetic resources. In general terms, this doctoral thesis covers pepper, eggplant and lettuce crops, and focuses on the agronomic, phenotypical and nutritional characterisation of the fraction of these resources, to finally be able to evaluate the most promising materials from the agronomic and nutraceutical points of view. The specific objectives of this research work are classified as follows:

1. Provide an agromorphological and phenotypical description of the autochthonous varieties of Valencian peppers so we can support lost genetic variability by contributing to promote and conserve the selected landraces **(Chapter 3)**

2. Analyse the nutritional and nutraceutical characteristics of the previously phenotyped pepper landraces (Chapter 3) to relate the health benefits that derive from their use as added value by considering two maturity stages **(Chapter 4)**

4. Re-evaluate 31 traditional eggplant varieties from the Valencian Community (Spain) to help to recover the biodiversity loss influenced by widely cultivated commercial hybrids. Firstly, detailed phenotypical data will be collected to finally determine the nutritional characteristics of the most promising landraces to gain be nefits that derive from their use **(Chapter 5)**

5. Evaluate the nutraceutical potential of lettuce landraces and commercial varieties in relation to their different morphologies and development stages (microgreens, baby leaves, adults) to establish the best health beneficial candidates in each compared scenario **(Chapter 6)**

6. Report the nutritional quality of lettuce landraces and commercial varieties in relation to their different morphologies and postharvest evolution in their nutrient composition to determine the best health benefit candidates throughout the market cycle **(Chapter7)**

CHAPTER 03

PHENOTYPIC DIVERGENCE AMONG SWEET PEPPER LANDRACES ASSESSED BY AGRO-MORPHOLOGICAL CHARACTERIZATION AS A BIODIVERSITY SOURCE

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Abstract
Traditional vegetable varieties constitute an important reservoir of biodiversity, so recovering, cultivating, and correctly classifying these landraces is part of key global heritage for the future of food security. In this study, 17 traditional pepper varieties from the Valencian Community (Spain) were characterized using 14 quantitative and 30 qualitative conventional morphological descriptors, including plant, flower, and fruit traits, in two ripening stages: green and red. As a result, landraces were grouped based mainly on their fruit morphology (G1: thin and elongated; G2: thick and robust; P-49: ball pepper). During a second trial, the preservation of the described characteristics was checked, and the number of fruit produced per plant was determined. From the acquired information, the most desirable traits that could be of interest for cultivation and harvesting practices were established, including erect growth habit, dense branching, big leaves, and uniformity and low persistence of fruit. Additionally, based mainly on fruit size and fruit wall thickness traits, the varieties with the highest potential to be marketed as fresh, P-37 (from G2), P-41, and P-72 (from G1), were determined. The ungrouped P-49 variety is an optimal candidate for industry processes because of its small size and robust fruit wall. The importance of phenotyping studies for preserving plant varieties is emphasized.

Keywords:
biodiversity; landrace; maturity stage; phenotype; pepper; trait; variety

1. INTRODUCTION

In relation to agriculture, the industrial revolution led the countryside to intensify agrarian mechanization, which triggered a reduction in self-sustenance economies [1–3]. Because of these outstanding changes, special importance started to be attached to high-yielding varieties, which, in addition to their high productivity, have uniform size and external fruit appearance. Their use was profitable for the food industry [1], and they were resistant to diseases [4]. This situation caused remarkable genetic erosion, which led to a loss of biodiversity in crop production [5]. Such was the impact of these changes that the FAO declared in 2004 that global crop biodiversity was a risk that could seriously compromise world food security. Unfortunately, this problem is still being faced.
Traditional varieties or landraces are those that have been differentiated throughout the ages in a certain ecogeographical area by attending to local edaphoclimatic conditions and traditional management and uses [6]. Landraces contribute to the development of the local economy by offering specific products, uses, and qualities that diversify the food base [2]. For this reason, recovering, cultivating, and correctly classifying traditional varieties form part of key global heritage for the future of food security and the economy by preserving biodiversity.

The Valencian Community (Spain) has an extensive heritage compound of very high diversity, with traditional vegetable varieties selected based on their organoleptic quality, by taking flavor as the prominent factor in seed selection [7,8], although many of them are in real danger of disappearing. As the importance of recovering biodiversity is known, it is necessary to encourage the general conservation of these varieties by promoting their cultivation in their areas of origin, recovering their commercial exploitation, and maintaining farmers' profitability and sustainability [6,7,9]. To carry out such conservation, a detailed description of the varieties' characteristics and quality requirements must be provided, in addition to all the wisdom acquired from practical experience in cultivation and reproduction [10]. Many characterization studies based on standardized morphological and agronomic descriptors developed by the International Board for Plant Genetic Resources (IBPGR, 1995) have been performed in peppers [11–15] and have demonstrated that they can contribute to optimizing plant breeding programs by providing helpful information for pepper breeders. According to Uddin et al. [16], clustering accessions in different groups may be useful for providing a basis for further crop improvement.

Pepper ***(Capsicum spp.)*** belongs to the Solanaceae family and is the second most-consumed vegetable worldwide, with 1.99 million hectares (ha) currently reserved for cultivation [17]. In Spain [18], pepper occupied 20,388 ha in 2017, cultivated mainly in the Mediterranean Region, where its considerable agronomic and economic importance makes it a relevant crop. Peppers provide important nutritional benefits, which make them even more valuable. Peppers' high antioxidant capacity, together with being very rich in ascorbic acid, carotene, phenols, xanthophylls, and flavonoids, make it a functional food [19,20]. Nonetheless, the proportion of these elements clearly depends on cultivar genotype and maturity stage, among other factors [21].

2. MATERIALS AND METHODS

2.1. Plant material

Plant material consisted of 17 pepper landraces (***C. annuum*** L.) that have never been characterized before, representing one part of the pepper germplasm collection from the Valencian Community (Figure 1). Accessions were provided by the Germplasm Banks of the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV, Spain) and the Valencian Institute of Agrarian Research (IVIA, Spain). They represent the most valued pepper types in the Valencian Community; a wide variety of phenotypes is managed. This trial forms part of a wider project in which we attempt to value traditional Valencian varieties, after previously publishing other articles such as Martínez-Ispizua et al. [22,23]. They have been previously selected based on geographical localization throughout the territory, fruit morphology, and possible use (Figure 1). The internal numerical code, passport identification from germplasm banks, group number based on fruit shape, fruit shape description, and origin for each accession are indicated in Table 1

2.2. Greenhouse Conditions

Two different experiments were run from May to September for two consecutive years in an unheated plastic multi-span greenhouse in the experimental field of the IVIA (Moncada, Valencia, Spain; 39°35′22.3″ N, 0°23′44.0″ W, 37 cm above sea level). The soil composition within a 20 cm depth was 68% sand, 11% clay, and 21% silt (sandy clay loam) and contained 0.61% organic matter, 0.051% total N, less than 8 mg kg−1 of P, 301 mg kg−1 of K, and 2.87 meq·100 g−1 of assimilable Mg. Soil electrical conductivity was 0.290 dS m−1 with a pH of 8.1.

Abbreviation Code	Germplasm Code	Group	Fruit Description	Origin/Cultivated Zone [3]	Intended Use (Consumption)
P-35	BGV005087 [1]	G2	Rectangular shape, blocky and with four shoulders and locule marked	Fanzara, Castellón, Spain	Roasted, preferably when mature (red)
P-36	BGV005035 [1]	G2	Irregular, rectangular-conical shape, but an inconsistent pattern, slightly marked shoulders	Chelva, Valencia, Spain	Roasted
P-37	BGV005097 [1]	G2	Triangular shape and apex truncated	Castillo de Villamalefa, Castellón, Spain	Roasted, preferably when mature (red)
P-39	BGV005115 [1]	G2	Triangular shape and apex truncated	Alicante, Spain	Roasted, preferably when mature (red)
P-40	BGV005125 [1]	G2	Rounded- elongated triangular shape and apex truncated	Elda, Alicante, Spain	Roasted, preferably when mature (red)
P-41	BGV014141 [1]	G1	Elongated (horn type), only slightly marked shoulders	Vinaroz, Castellón, Spain	Fried, preferably when immature (green)
P-42	BGV014145 [1]	G1	Elongated (horn type), only slightly marked shoulders	Almenara, Castellón, Spain	Fried, preferably when immature (green)
P-44	BGV016188 [1]	G1	Elongated (horn type), only slightly marked shoulders	Guardamar del Segura, Alicante, Spain	Fried, preferably when immature (green)
P-45	BGV005064 [1]	G2	Triangular shape and apex truncated	Ademuz, Valencia, Spain	Roasted, preferably when mature (red)
P-46	BGV005085 [1]	G2	Rectangular shape, blocky and with four shoulders and locule marked	Onda, Castellón, Spain	Roasted
P-47	BGV005040 [1]	G2	Rounded-elongated triangular shape and apex truncated	Siete Aguas, Valencia, Spain	Roasted
P-48	BGV005034 [1]	G1	Elongated (horn type), only slightly marked shoulders	Chelva, Valencia, Spain	Fried, preferably when immature (green)
P-49	BGV005046 [1]	-	Ball-like shape with only slightly marked shoulders	Benissa, Alicante, Spain	Roasted, preferably when mature (red)
P-50	BGV005116 [1]	G2	Triangular shape and apex truncated	Rojales, Alicante, Spain	Roasted
P-51	BGV014553 [1]	G2	Rectangular shape, blocky and with four shoulders and locule marked	Tales, Castellón, Spain	Roasted
P-70	IVIA 70 [2]	G2	Rounded- elongated triangular shape and apex truncated	Moncada, Valencia, Spain	Roasted, preferably when immature (green)
P-72	IVIA 72 [2]	G1	Elongated (horn type), only slightly marked shoulders	Canal de Navarrés, Valencia, Spain	Fried, preferably when immature (green)

Table 1. Abbreviation, germplasm collection code, group (based mainly on fruit characteristics obtained in the actual study, G1 = elongated; G2 = triangular, square or blocky; "-" = non-grouped), fruit shape description, and origin of the 17 varieties of pepper used in the study. Plant material was provided by: (1) the Valencian Institute for the Conservation and Improvement of Agrobiodiversity (COMAV, Spain) and (2) the Valencian Institute for Agricultural Research (IVIA, Spain). (3) Town, province, country.

*** Figure 1:** Pepper fruit from the 17 pepper landraces in two different ripening stages: immature: green; mature: red. The surface of each section making up the grid on which photos were taken equals 1 cm2.

2.3. Phenotyping Study

The morphologic characterization was determined during the first-year experiment. The seeds from the 17 pepper landraces were sown on 5 March 2019. Seedlings were transplanted on 2 May 2019. Then, 2-month-old plants were grown under greenhouse conditions in single rows set 110 cm apart by leaving 50 cm between each plant. Eight plants per landrace were selected for this purpose. All eight pepper plants corresponding to the same variety were planted consecutively in a single cultivation area inside the greenhouse. The spot for each variety was selected randomly. Plant irrigation met 100% crop evapotranspiration, as described in Penella et al. [24], with a drip system. Nutrients were applied through the irrigation system at a rate (kg ha-1) of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO, and 35 MgO, as recommended by Maroto [25]. The average range of the minimum and maximum temperatures during the first-year experiment was 12–24 °C for May, 15–28 °C for June, 19–32 °C for July, 19–32 °C for August, and 18–29 °C for September [26].

Phenotypic measurements were taken upon flowering, except for fruit traits (upon commercial maturity, from July to September, depending on the descriptor and landrace). The data for the plant, leaf, and flower traits were acquired from eight independent plants per landrace. Fruit traits were measured in 10 different representative fruit per landrace and maturity state (green and red). Phenotyping traits were evaluated on plants using the International Board for Plant Genetic Resources descriptors for pepper [27] as a basis. The quantitative and qualitative descriptors data are recorded in Table S1.

2.4. Agronomic Trait

During the second-year experiment, the number of fruits produced per plant was evaluated after verifying that the phenotype characteristics described during the first-year experiment remained for each landrace. The seeds from the same landraces were sown on 7 March 2020, and the seedlings (4 plants per landrace) were planted on 13 May 2020. Like the year before, all four pepper plants corresponding to the same variety were planted consecutively in a single cultivation area inside the greenhouse. The spot for each variety was selected randomly. Agronomic culture practices were similar to those of the first year. The average range of the minimum and maximum temperatures was 11–31 °C for May, 14–31 °C for June, 18–33 °C for July, 19–34 °C for August, and 15–32 °C for September [20].

Fully developed fruit were harvested on four consecutive dates from the beginning of July to the end of August (green fruit: 10 July and 23 July; green and red fruit: 5 August and 24 August). The results are presented as the number of fruit per plant-1 (No. plant-1).

2.5. Statistical Analysis

For quantitative traits, a one-way ANOVA analysis was performed with the results obtained from the evaluated parameters with Statgraphics Centurion XVII (Statistical Graphics Corporation 2014, Englewood Cliffs, NJ, USA)

using the selected landraces as the factor of analyses. The results were expressed as the means and accepted as being significantly different at the 95% confidence interval ($p \leq 0.05$). For qualitative traits, the mean value for each landrace was represented by the most frequent representation of the trait after classifying the independent samples of plants and fruits (n = 8 for vegetative traits and n = 10 for fruit traits) according to the scale (Table S1). These values were used to calculate the frequency distribution of the traits for the 17 landraces.

Principal component analysis (PCA) was performed for standardized values using pairwise Euclidean distances among the means of accessions to determine similarities between genotypes. The extracted and statistically significant eigenvalues (1≤) and the relative and cumulative proportions of total variance explained by the first four components were calculated. A two-dimensional (2D) scatter plot (first vs. second PC) was executed based on a distance matrix for the principal components to visualize the relation explaining the traits. Information extracted from the feature plot was incorporated into the scatter plot to highlight the traits that contributed the most to the variability among landraces. PCA parameters were estimated using the ggrepel, Stat, FactoMineR, and Factoextra R packages. As complementary information, landraces were clustered and represented in a dendrogram (Figure S5) with Statgraphics Centurion XVII to further understand the relations found between the varieties.

A correlation analysis was run with the selected quantitative plant, leaf, and fruit traits, in which the individual samples of each landrace (n = 17) were subjected to linear regression and correlation coefficients (r) were obtained. Values were accepted as being significantly different at the 95% confidence interval ($p \leq 0.05$). In addition, a separate correlation network was built [28] to help understand how different conventional descriptors contributed to diversity. The illustration was obtained using Comprehensive R Archive Network, using the Corrplot R package.

3. RESULTS

3.1. Phenotypic Differences in Quantitative Vegetative Traits

Table 2 offers all the collected quantitative data about the vegetative parts and flowers of the pepper plants and their statistics. The mean of the group, range, CV, and significance are presented in Table S2. Generally, for the characters related to plant dimensions, the G1 landraces developed statistical significances in three length traits: stem to first bifurcation, plant width, and leaf length. In stem to first bifurcation, landraces P-44 and P-72 stood out for having the highest values (mean 33.1 ± 1.1 cm, 19.5% over the G1 average), while the opposite trend was observed in P-41 and P-48 (mean of 22.2 ± 0.4, 20% below the G1 average). Landraces P-41, P-48, and P-72 stood out for their plant width (mean of 67 ± 8.5, 10% above the G1 average). The highest leaf length values were for P-42, P-44, and P-72 (19.1 ± 0.9 cm), while the lowest ones went to P-41 and P-48 (16.9 ± 0.2 cm).

Group	Landrace	L (cm)		W (cm)		L/W		Pedicel L (cm)		Wall Thickness (mm)		Locule number		FW (g)		DW (%)	
G1	P-41	20.9	a	3.7	b	5.9	a	3.8	b	3.1	a	2.7	b	96.7	a	9.8	c
	P-42	23.0	ab	4.7	a	5.0	ab	4.4	a	3.5	a	3.4	a	100.8	a	8.8	bc
	P-44	16.2	a	3.0	b	5.5	a	4.2	ab	2.5	b	2.9	ab	62.7	bc	10.8	b
	P-48	13.2	b	3.2	b	4.5	b	2.8	c	2.7	b	3.4	a	59.1	c	13.8	a
	P-72	16.6	ab	3.0	b	5.1	ab	4.7	a	3.3	a	2.6	b	84.6	ab	9.8	bc
G2	P-35	9.7	d	7.0	bc	1.4	e	3.4	bc	4.8	cde	3.6	a	95.9	c	9.4	bcde
	P-36	11.3	abcd	5.3	d	2.0	bc	3.9	ab	3.6	g	3.4	a	122.0	ab	10.2	abc
	P-37	11.7	ab	7.6	bc	1.5	de	3.3	bcd	4.1	efg	3.3	a	107.9	bc	10.8	ab
	P-39	9.5	cd	5.5	d	1.8	cd	3.9	ab	4.0	defg	3.8	a	110.2	abc	11.2	a
	P-40	9.6	d	5.6	d	1.8	c	2.8	d	4.7	cde	3.7	a	118.0	abc	10.7	ab
	P-45	11.6	ab	8.2	a	1.4	e	4.2	a	8.7	b	3.6	a	136.3	a	9.6	abcd
	P-46	10.4	bcd	7.8	ab	1.4	ef	3.4	bc	4.3	def	3.7	a	135.2	a	8.3	de
	P-47	12.6	a	5.5	d	2.3	a	4.2	a	4.8	cd	3.6	a	100.1	bc	9.5	abcde
	P-50	11.7	abc	6.4	cd	1.8	c	3.9	ab	9.8	a	3.3	a	115.4	abc	8.4	cde
	P-51	9.6	d	8.4	a	1.1	f	3.1	cd	5.2	c	3.4	a	113.3	abc	8.7	cde
	P-70	12.7	a	5.7	d	2.3	ab	4.2	a	3.9	fg	3.3	a	97.9	c	7.6	e
-	P-49	5.7		6.7		0.9		3.2		8.2		3.3		86.1		10.9	

*** Table 2.** Quantitative traits for conventional morphologic descriptors in plant, leaf, and flower of 17 local pepper landraces cultivated in Spain. Statistics were performed by the formed groups based on fruit shape; G1 = elongated; G2 = triangular, square or blocky. Data belonging to the outlier ungrouped landrace (-, P-49 landrace) are also shown. For each landrace, values represent the mean for the studied conventional morphological descriptors (n = 8). Different letters in a group indicate significant differences at $p \leq 0.05$ (LSD test). L: length; W: width; FW: fresh weight; DW: dry weight.

In contrast, all the plant quantitative traits showed significant differences in the G2 landraces. Regarding stem-related characters, landrace P-40 stood out for being much longer than the rest (43 ± 2.1 cm, 47.8% over the G2 average) when taking into account distance to the first bifurcation point. However, it had one of the smallest stem diameters (14.4 ± 0.8 cm, 10.8% below the G2 average). Finally, P-50 was outstanding for the large diameter of its stem (25.4 ± 5.1 cm, 56.6% higher than the G2 average). Landraces P-35, P-36, P-37, P-40, P-45, P-46, and P-51 stood out for developing the widest plants (mean 67.9 ± 2.7 cm, 7.7% over the G1 average). The thinnest plants were recorded for P-39, P-47, P-50, and P-70 (mean 54.4 ± 3.0 cm, 13.7% below the G2 average). Regarding leaves, landrace P-40 was remarkable for its big leaf size (23.2 ± 1.9 cm long and 14.8 ± 1.4 cm wide), followed by P-39 and P-45, whose leaves were also as long as P-40 but narrower (mean 22.3 ± 0.9 cm long and 12.2 ± 0.7 cm wide). P-35 and P-50 had the shortest leaves (mean 17.3 ± 0.9 cm). However, 5 of the 11 G2 accessions (P-35, P-36, P-37, P-50, P-70) appeared to have a similar leaf width (mean 10.2 ± 0.2 cm) with a downward trend. Two landraces were highlighted for the number of flowers per axile, P-37 and P-45, with 51.5% and 73.5% more blooms, respectively, than the G2 mean value (1.32 flowers per axile).

Finally, the ungrouped landrace, P-49, stood out for stem distance to first bifurcation (22.5% and 16.6% higher than the G1 and G2 means, respectively) and its narrow plants (9.8% and 5.6% lower than the G1 and G2 means, respectively).

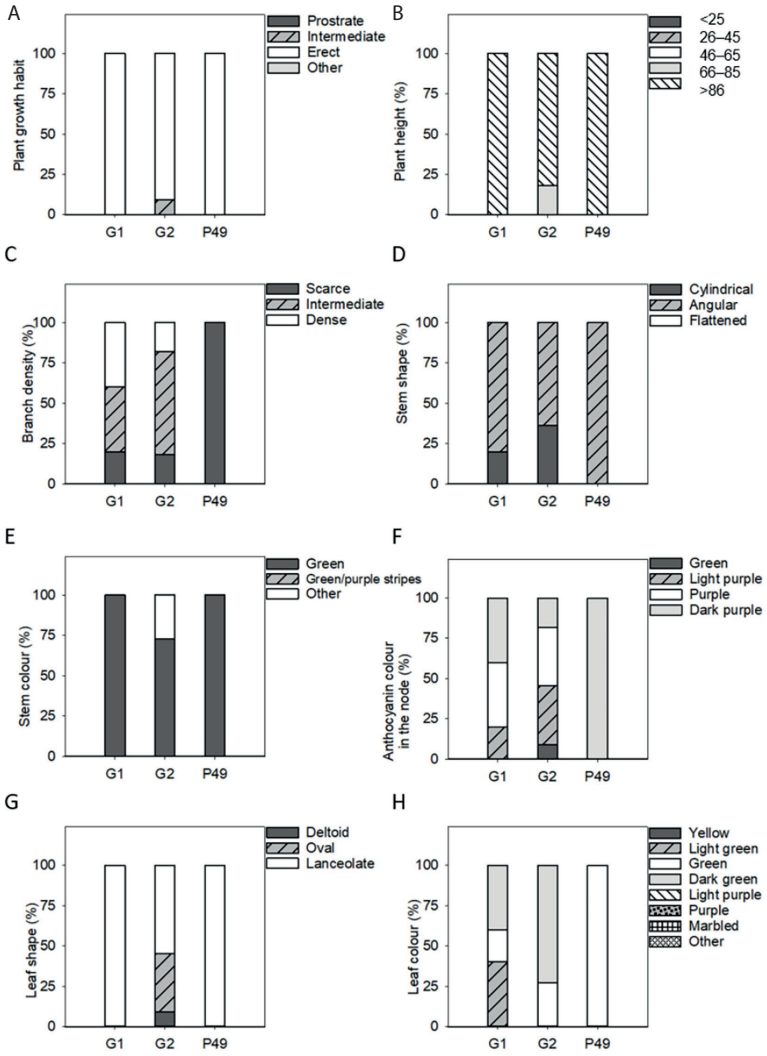
3.2. Phenotypic Differences in Qualitative Vegetative Traits

Many of the 17 studied pepper landraces generally developed erect growing plants (16 out of 17) (Figure 2A), which makes it easier for them to grow to a height exceeding 86 cm (15 out of 17) (Figure 2B).

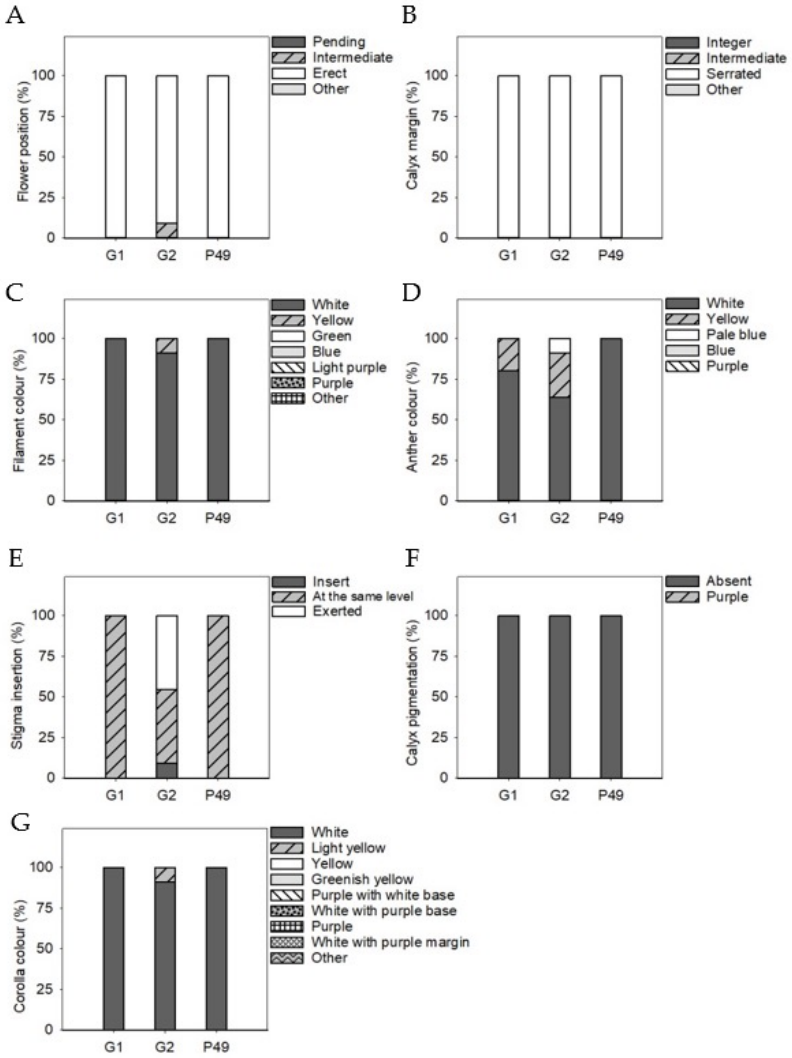
For G1, no clear trend explained branch density (Figure 2C). However, almost all the landraces (4 out of 5) developed angular green stems (Figure 2D,E). Anthocyanin intensity in nodes varied from dark to light purple (Figure 2F). Leaf shape was clearly lanceolate and green in color. Some variation in intensity was noted and ranged from light (2 out of 5 landraces) to dark (2 out of 5 landraces) green (Figure 2G,H) in G1. Most G2 plants presented intermediate branch density and angular or cylindrical stems (Figure 2C,D). Although most stems were green with purple or light purple nodes (Figure 2E,F), one landrace had green nodes (P-47) and two had dark purple ones (P-37 and P-46). Leaves were mostly lanceolate or oval (5 and 4 out of 11, respectively), and their main color was dark green (3 out of 11) (Figure 3G,H).

P-49 presented erect plants that were more than 86 cm tall with scarce branch density. Stems were angular and green with dark purple nodes, and leaves were clearly lanceolate and green.

All the G1 flowers and P-49 (Figure 3) were erect, with a serrated calyx margin and white-yellow anthers. The stigma was inserted at the same height as anthers (Figure 3E); the calix was not pigmented, and the corolla was white. In G2, flowers were mostly erect (10 out of 11), with a serrated calyx margin (11 out of 11) and their own white filaments (10 out of 11) and anthers (7 out of 11). Their stigma appeared inserted at the same height as anthers. The calyx was not pigmented, and the corolla was white in all the studied cases (10 out of 11).



*** Figure 2:** Frequency distribution of the plant, stem, and leaf qualitative traits in 17 pepper landraces.(A) Plant growth habit; (B) Plant height; (C) Branch density; (D) Stem shape; (E) Stem colour; (F) Anthocyanin colour in the node; (G) Leaf shape; (H) Leaf colour.The mean value for each landrace was represented by the most frequent representation of the trait after classifying the independent plant samples (n = 8) according to the scale (Table S1).



*** Figure 3.** Frequency distribution of the flower qualitative traits in the 17 pepper landraces. (A) Flower position; (B) Calyx margin; (C) Filament colour; (D) Anther colour; (E) Stigma insertion; (F) Calyx pigmentation; (G) Corolla colour. The mean value for each landrace was represented by the most frequent representation of the trait after classifying the independent plant samples (n = 8) according to the scale (Table S1).

3.3. Phenotypic Differences in Quantitative Fruit Traits

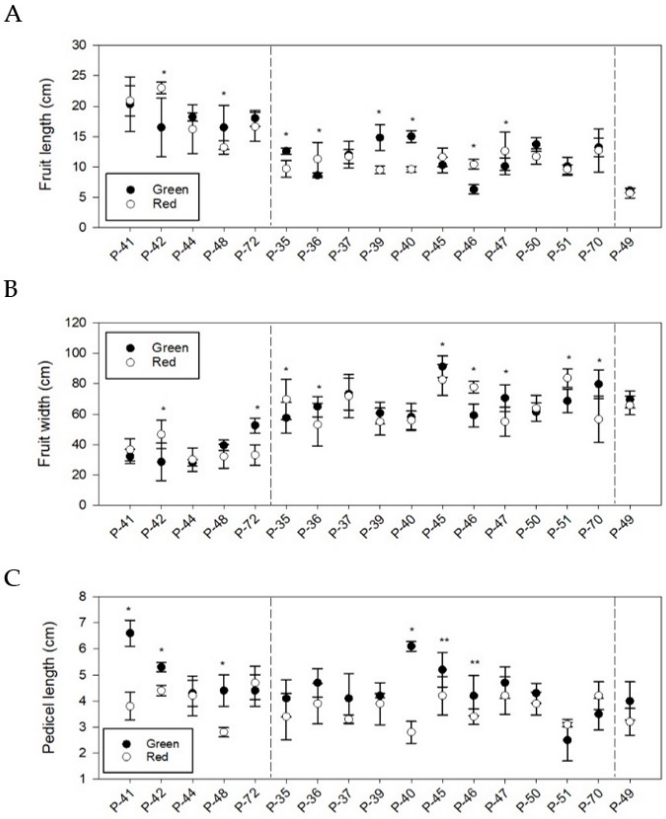
All the quantitative data describing the green and red fruit are found in Tables 3 and 4, respectively, along with the differences found between landraces for each trait in all the fruit groups. Once again, group average, range, CV, and significance are presented in Table S2. Regarding fruit dimensions, longer fruit were also narrower, and vice versa. In the G1 landraces, the difference between length and width in fruit P-41 and P-44 was independent of maturity state. They had the highest L/W (6.4 ± 0.1 and 5.3 ± 0.2 in the green and the red fruit, respectively). On the contrary, the lowest L/W was observed in the P-72 green fruit (3.5) because of its high width value (5.3 cm, 45.8% over the G1 mean). The opposite trend was noted upon ripening (3.0 cm wide, 5.6% below the mean) with a high L/W value (5.1). When comparing both states (Figure 4A,B), general behavior involved maintained dimensions, but the P-42 red fruit were statistically longer and wider than the green ones, while P-48 was wider in the immature state.

Group	Landrace	Length (cm)		Width (cm)		L/W		Pedicel L (cm)		Wall Thickness (mm)		Locule Number		FW (g)		DW (%)	
G1	P-41	20.3	a	3.2	c	6.4	a	6.6	a	3.0	b	2.5	b	84.9	bc	6.7	bc
	P-42	16.5	b	2.9	c	6.4	a	5.3	b	2.1	c	2.8	b	89.0	ab	5.8	d
	P-44	18.2	ab	2.8	c	6.5	a	4.3	c	3.2	b	3.5	a	71.2	c	7.0	b
	P-48	16.5	b	3.9	b	4.4	b	4.4	c	2.8	b	3.0	ab	74.6	bc	8.5	a
	P-72	18.0	ab	5.3	a	3.5	b	4.4	c	4.3	a	2.6	b	100.9	ab	6.3	c
G2	P-35	12.6	bc	5.8	f	2.4	b	4.1	cde	4.3	d	3.5	bc	135.6	bc	6.1	bc
	P-36	8.6	e	6.5	def	1.4	de	4.7	bc	4.3	d	3.0	d	159.4	ab	6.2	b
	P-37	12.0	cd	7.3	bc	1.5	cd	4.1	de	4.5	cd	3.0	cd	107.6	e	6.2	b
	P-39	14.8	a	6.1	f	2.3	b	4.2	cde	4.3	cd	3.5	bcd	145.7	abc	6.8	a
	P-40	15.0	a	5.8	f	2.7	a	6.1	a	4.2	d	3.8	ab	131.8	cd	5.8	bcd
	P-45	10.3	d	9.1	a	1.1	e	5.2	b	5.3	ab	4.0	a	157.6	abc	5.8	bcd
	P-46	6.3	f	5.9	f	1.1	e	4.2	cd	4.4	cd	3.8	a	172.8	a	5.5	d
	P-47	10.1	d	7.1	cd	1.5	cd	4.7	bcd	5.0	abc	4.0	a	134.3	bcd	5.7	cd
	P-50	13.7	ab	6.1	ef	2.3	b	4.3	cd	5.0	bcd	3.0	d	135.1	bc	5.9	bcd
	P-51	10.1	d	6.9	cde	1.5	cd	2.5	f	4.6	cd	3.5	b	139.1	bc	5.5	d
	P-70	13.2	bc	8.0	b	1.7	c	3.5	e	5.6	a	3.5	b	143.4	bc	5.5	d
-	P-49	6.1		7.0		0.9		4.0		7.1		2.8		124.7		6.3	

*** Table 3.** Quantitative traits for conventional morphologic descriptors in green fruits of the 17 local pepper landraces cultivated in Spain. Statistics were performed by the formed groups based on fruit shape; G1 = elongated; G2 = triangular, square or blocky. Data belonging to the outlier ungrouped landrace (-, P-49 landrace) are also shown. For each landrace, values represent the mean for the studied conventional morphological descriptors (n = 10). Different letters in a group indicate significant differences at $p \leq 0.05$ (LSD test). L: length; W: width; FW: fresh weight; DW: dry weight.

Group	Landrace	L (cm)		W (cm)		L/W		Pedicel L (cm)		Wall Thickness (mm)		Locule number		FW (g)		DW (%)	
G1	P-41	20.9	a	3.7	b	5.9	a	3.8	b	3.1	a	2.7	b	96.7	a	9.8	c
	P-42	23.0	ab	4.7	a	5.0	ab	4.4	a	3.5	a	3.4	a	100.8	a	8.8	bc
	P-44	16.2	a	3.0	b	5.5	a	4.2	ab	2.5	b	2.9	ab	62.7	bc	10.8	b
	P-48	13.2	b	3.2	b	4.5	b	2.8	c	2.7	b	3.4	a	59.1	c	13.8	a
	P-72	16.6	ab	3.0	b	5.1	ab	4.7	a	3.3	a	2.6	b	84.6	ab	9.8	bc
G2	P-35	9.7	d	7.0	bc	1.4	e	3.4	bc	4.8	cde	3.6	a	95.9	c	9.4	bcde
	P-36	11.3	abcd	5.3	d	2.0	bc	3.9	ab	3.6	g	3.4	a	122.0	ab	10.2	abc
	P-37	11.7	ab	7.6	bc	1.5	de	3.3	bcd	4.1	efg	3.3	a	107.9	bc	10.8	ab
	P-39	9.5	cd	5.5	d	1.8	cd	3.9	ab	4.0	defg	3.8	a	110.2	abc	11.2	a
	P-40	9.6	d	5.6	d	1.8	c	2.8	d	4.7	cde	3.7	a	118.0	abc	10.7	ab
	P-45	11.6	ab	8.2	a	1.4	e	4.2	a	8.7	b	3.6	a	136.3	a	9.6	abcd
	P-46	10.4	bcd	7.8	ab	1.4	ef	3.4	bc	4.3	def	3.7	a	135.2	a	8.3	de
	P-47	12.6	a	5.5	d	2.3	a	4.2	a	4.8	cd	3.6	a	100.1	bc	9.5	abcde
	P-50	11.7	abc	6.4	cd	1.8	c	3.9	ab	9.8	a	3.3	a	115.4	abc	8.4	cde
	P-51	9.6	d	8.4	a	1.1	f	3.1	cd	5.2	c	3.4	a	113.3	abc	8.7	cde
	P-70	12.7	a	5.7	d	2.3	ab	4.2	a	3.9	fg	3.3	a	97.9	c	7.6	e
-	P-49	5.7		6.7		0.9		3.2		8.2		3.3		86.1		10.9	

*** Table 4.** Quantitative traits for conventional morphologic descriptors in red fruit of the 17 local pepper landraces cultivated in Spain. Statistics were performed by the formed groups based on fruit shape; G1 = elongated; G2 = triangular, square or blocky. Data belonging to the outlier ungrouped landrace (-, P-49 landrace) are also shown. For each landrace, values represent the mean for the studied conventional morphological descriptors (n = 10 for fruit traits). Different letters in a group indicate significant differences at $p \leq 0.05$ (LSD test). L: length; W: width; FW: fresh weight; DW: dry weight.



*** Figure 4:** Morphological ((A): length; (B): width; (C): pedicel length) traits of the fruit of the 17 pepper landraces in two ripening stages. Values are the mean ± SD of n = 10 fruit. Asterisks indicate significant differences of the red fruit to the green fruit for each landrace ($p < 0.05$).

In the G2 green fruit (Table 3), the highest L/W values were for landraces P-35, P-39, P-40, and P-50 (2.4 ± 0.2), given their long narrow dimensions (20.5% over and 12.3% below the mean, respectively). On the contrary, P-36 and P-46 presented short narrow fruits (26.1% and 8.4% below the G2 mean, respectively) associated with low L/Ws (1.2 ± 0.2). P-45 also stood out for its low L/W but obtained the widest fruit in both ripening stages (34.7% over the G2 mean). The highest L/W values upon maturity were for P-47 and P-70, given their long but narrow fruit (around 14.6% above and 17.7% below the G2 length and width means, respectively) (Table 4). When comparing maturity states (Figure 4A,B), three landraces (P-35, P-39, P-40) were outstanding for having statistically significant longer fruit when green, while fruit P-36, P-46, and P-47 were longer when red (Figure 4A,B).

By far, the least elongated fruits of all the landraces appeared for the P-49 variety (65.9% and 44.5% below the G1 and G2 means, respectively), which falls in line with the lowest L/W (around 0.88 in maturity states, 83.0% and 47.1% below the G1 and G2 means, respectively (Table 3).

When analyzing pedicel length (Figure 4C), the general trend was to develop shorter pedicels in the red than green fruit (20.7%, 16.3%, and 20.0% decrease in G1, G2, and P-49, respectively), but a statistical significance was seen in only six landraces (P-41, P-42, and P-48 from G1 and P-40, P-45, and P-46 from G2). According to groups (Tables 4 and 5), P48 (G1) was highlighted for short pedicel length in both maturity states, and P-40 (G2) stood out for having the longest pedicels when green but the shortest when red. The fruits of P-51 (G2) developed short pedicels independently of their ripening state.

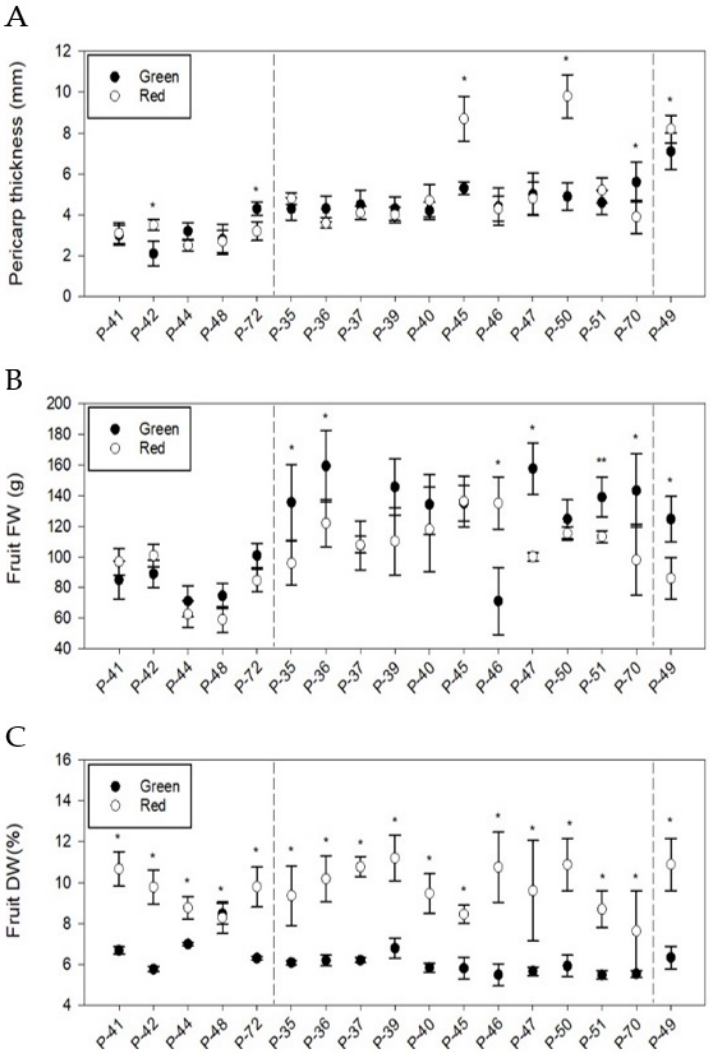
Group	Landrace	Number Plant^{-1}
G1	P-41	22.3
	P-42	24.3
	P-44	10.5
	P-48	19.8
	P-72	11.0
	Mean	17.6
G2	P-35	8.50
	P-36	16.00
	P-37	21.75
	P-39	11.75
	P-40	15.25
	P-45	16.00
	P-46	11.25
	P-47	13.75
	P-50	12.25
	P-51	17.00
	P-70	12.25
	Mean	14.2
-	P-49	10.3

*** Table 5:** Productivity parameter number of fruits per plant (n = 4) in the collection of the 17 pepper landraces cultivated in Spain.

Fruit wall thickness was studied. The landraces from G1 stood out for their low values in both maturity states (from 2.1 to 4.3 mm) compared to the G2 fruits, for which the mean values in the G2 fruit were 56.7% and 73.3% higher than the G1 green and red values, respectively. The ungrouped landrace (P-49) stood out for producing fruit with the thickest walls (136.7% and 173.3% higher than the G1 green and red fruits, respectively). In G2, 4 of the 11 landraces (P-45, P-47, P-50, P-70) had fruit wall thickness values above 5.0 cm in the green fruit, but they ranged from 4.2 to 4.6 in the others (Table 3). The maturity process significantly increased pericarp thickness in P-45 and P-50 (64.2% and 96.0%, respectively) but reduced it by 30% in P-70 (Figure 5A).

The majority of landraces had fruits formed by three independent locules (Tables 3 and 4). In certain varieties, having four locules was the general tendency (i.e., P-35, P-39, P-40, P-45, P-46, and P-47 from G2) or only two (P-41 and P-72 from G1).

Regarding fruit fresh weight, the G1 landraces had the lightest fruits in the assay, regardless of maturity state (Figure 5B). An opposite trend was observed in the fruits from G2 and P-49 because they were heavier in the green than in the red state (20% and 30.9% in G2 and P-49, respectively) (Figure 5B). In G1 (Tables 3 and 4), P-42 and P-72 were highlighted for their heavy fruit in both the green (95 ± 8.4 g, 12.9% above the G1 mean) and red (92.7 ± 11.5 g, 12.8% above the G1 mean) fruit. The lightest green fruit were from P-44 and P-48 (72.9 ± 2.4 g, 13.3% below the G1 mean), and these values were lowered to 60.9 ± 2.5 g (25.9% below the G1 mean) when red in color. Of all the G2 landraces (Table 3), the heaviest green fruit were recorded in landraces P-36, P-39, P-45, and P-46 (158.9 ± 11.1 g, 11.9% above the G2 mean), while the lightest ones went to P-37 (107.6 g, 24.2% below the G2 mean). In G2, six landraces (P-35, P-36, P-46, P-47, P-51, P-70) showed statistical differences between green and red fruit (Figure 5B).

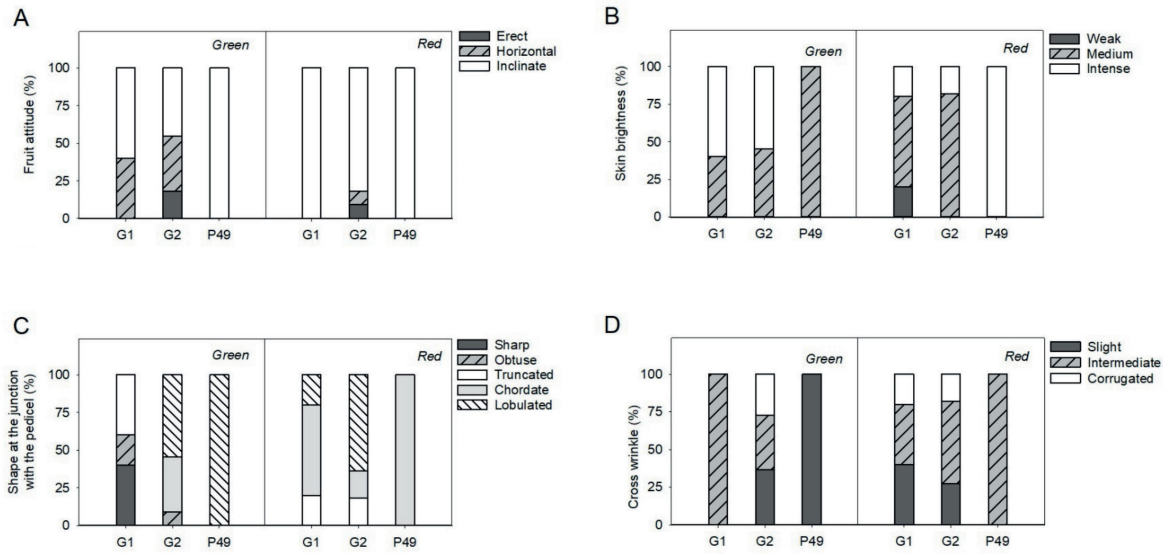


*** Figure 5:** Biomass traits ((A): pericarp thickness; (B): fresh weight; (C): dry weight) of the fruit of the 17 pepper landraces in two ripening stages. Values are the mean ± SD of n = 10 fruit. Asterisks indicate significant differences of the red fruit to the green fruit for each landrace ($p < 0.05$).

As a general trend, the DW percentage considerably increased in the mature fruit (55.6%, 61.0%, and 73.0% in G1, G2, and P-49, respectively) (Figure 5C). In G1, the highest value for this trait was recorded for landrace P-48 in the green state (25.0% over the G1 green mean). In G2, landrace P-39 obtained the highest DW percentage in the green fruit (0.9% over the G2 green mean), while this ratio increased to 6 out of 11 (P-36, P-37, P-39, P-40, P-45, P-47) when analyzed in the red fruit (10.3 ± 0.7%, 0.8% over the G2 red mean) (Table 4). For the number of fruit produced per plant (Table 5), the G1 mean had the highest total values. The G1 varieties that produced the fewest peppers were P-44 and P-72 (10.5 and 11 fruit plant-1, respectively), while landrace P-42 stood out for producing many (56% more fruit than the G1 mean). Of the G2 landraces, P-37 produced the most fruit plant-1 (63.3% more fruit than the G2 mean). P-49 did not produce much fruit compared to the production averages obtained in the other variety groups (41.5% and 27.5% fewer fruit than the G1 and G2 means, respectively).

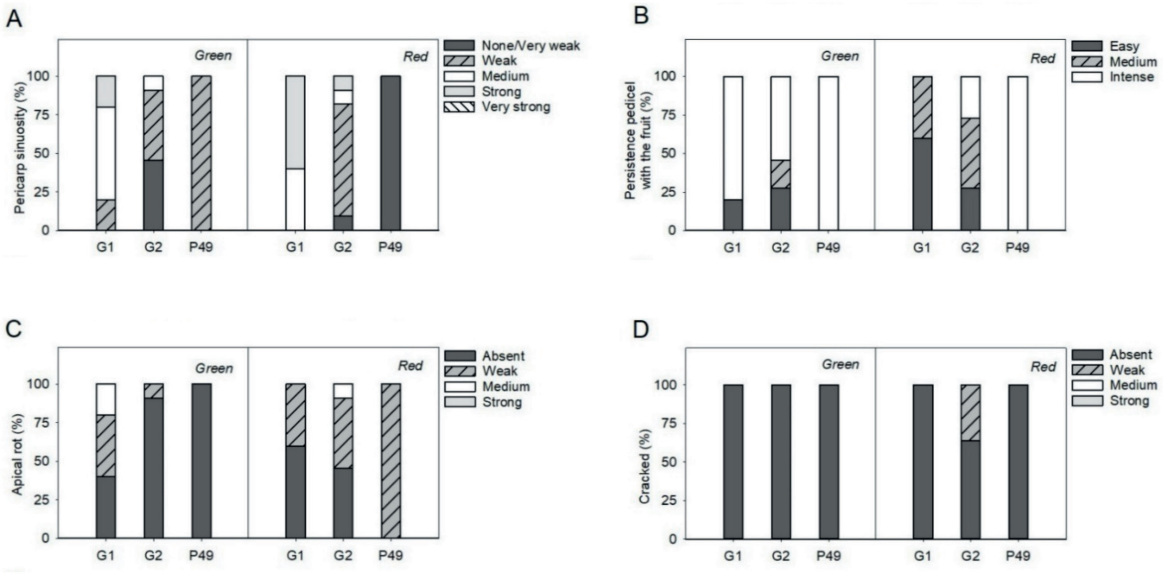
3.4. Phenotypic Differences in Qualitative Fruit Traits

The frequency of several qualitative fruit traits occurring for each group and maturation stage was recorded in several histograms (Figures 6, 7, S1, and S2).



*** Figure 6:** Frequency distribution of the selected fruit qualitative traits in the 17 pepper landraces in two ripening stages (green and red); (A) fruit attitude, (B) skin brightness, (C) shape at the junction with the pedicel, (D) cross wrinkle. The mean value for each landrace was represented by the most frequent representation of the trait after classifying the independent fruit samples (n = 10) according to the scale (Table S1).

The G1 group fruit presented horizontal or inclined attitude (Figure 6A) and medium or intense skin brightness (Figure 6B) and were characterized by their elongated fruit shape (Figure S1A). The junction with the pedicel (Figure 6C) changed its sharp truncated shape to become more chordate with maturity. G2 was composed of varieties whose fruit had different morphologies (blocky, triangular, flared) (Figure S1A). Maturation changed somewhat in them because it distorted their attitude (Figure 6A) and skin brightness (Figure 6B) by reducing morphology (Figure 6C), which became more flared and shaped at the junction with the pedicel and was mainly chordate or lobulated in the green fruit but mostly lobulated or even truncated in the red fruit. The P-49 fruit had an inclined attitude (Figure 6A); skin brightness was amplified with maturity (Figure 6B), and they were the only ones with a round shape (Figure S1A). Maturation changed their shape at the junction with the pedicel (Figure 6C) to become lobulated when green and chordate when red.



*** Figure 7:** Frequency distribution of the selected fruit qualitative traits in the 17 pepper landraces in two ripening stages (green and red); (A) pericarp sinuosity, (B) persistence pedicel with fruit, (C) apical rot, (D) cracked. The mean value for each landrace was represented by the most frequent representation of the trait after classifying the independent fruit samples (n = 10) according to the scale (Table S1).

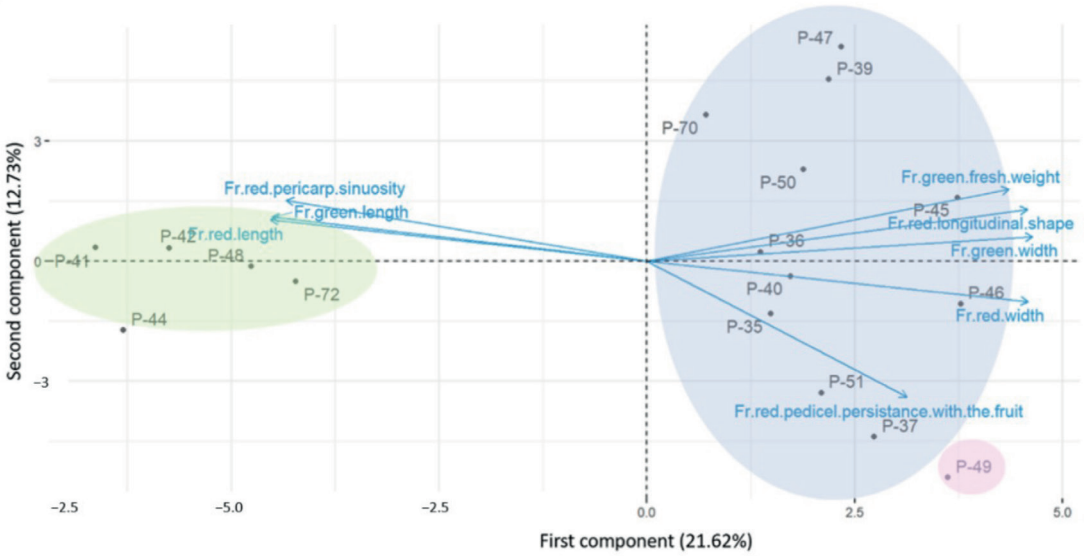
G1 and G2 fruit were less wrinkled upon maturity (Figure 6D), but pericarp sinuosity increased (Figure 7A). Pedicel persistence with both the stem and, especially, fruit reduced during maturation (Figures 7B and S2B). Maturity appeared to be related to the increased appearance of apical rot (Figure 7C), especially in the G2 fruit, for which the number of affected landraces rose from 1 to 6. This also occurred in P-49. Finally, fruit cracking (Figure 7D) seemed to accentuate with ripening, but only in G2 (rising from 0 to 5 out of 11 in the red fruit).

3.5. PCA Analysis

The PCA analysis and eigenvalues higher than 1 reflected different patterns in the pepper landraces correlation (Tables S3 and S4). Fifteen principal components were determined, which described around 98.86% of the total variability between landraces. Only the landrace distributions based on the strongest principal components are herein shown; the first (Figure S3), second, third, and fourth components of the PCA, respectively, accounted for 21.6%, 12.7%, 9.6%, and 8.7% of the variance of the total variation in the studied traits, while cumulative variance explained 52.71% (Tables S3 and S4). Only the correlations with an absolute value of ≥0.150 were listed to simplify the results (Tables 2 and S4) and to highlight only those parameters that were really important for establishing differences between varieties.

Pepper landraces were widespread over the PCA projection area (Figures 8 and S4). In general, the landraces that were similar in fruit shape were placed together, which suggests the importance of fruit morphology-related traits. Two groups were arranged based on their fruit length (L) and width (W) dimensions (G1 = elongated, $L/W > 5$; G2 = triangular, square or blocky, $1 < L/W < 5$). Landrace P-49 was not included in any of these groups because of its unique fruit morphology, round shape ($L/W < 1$), and some distinctive characteristics (related to the percentage of dry weight, placenta length), and was, therefore, somewhat marginalized in the lower part of the plot (Figure 2).

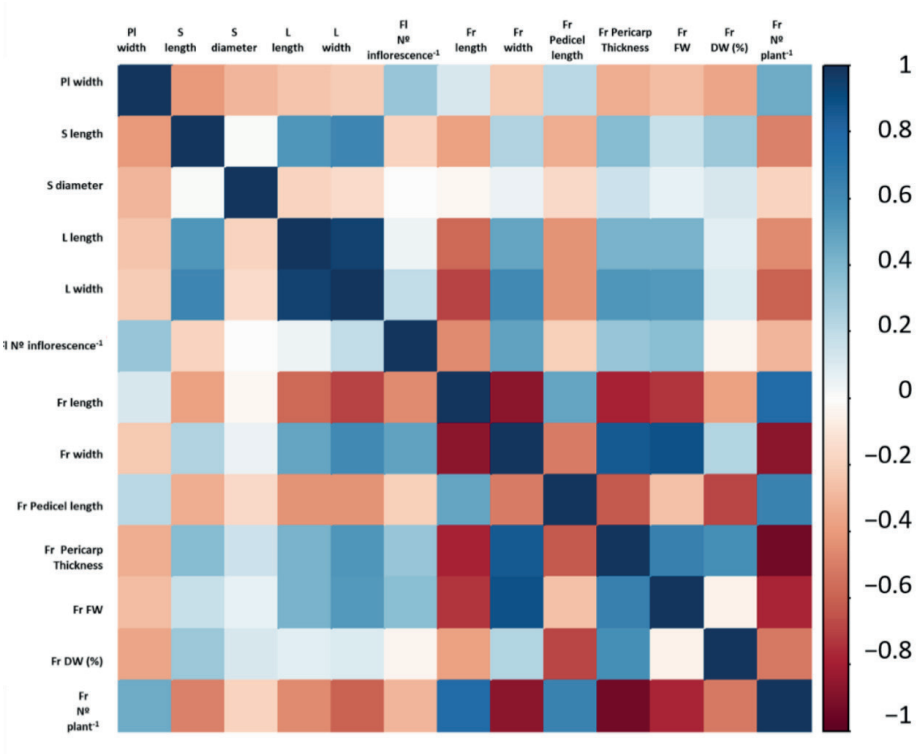
In the plot corresponding to the first and second components (Figure 8), the G1 varieties (P-41, P-42, P-44, P-48, and P-72) are located furthest to the left and stand out for developing the most elongated and narrow fruit in the study and for having the lightest fruit with the strongest pericarp sinuosity. Based on apex shape, they can be classified as pointed. In contrast, the G2 landraces stand out for developing shorter, heavier, and wider fruit than the G1 ones. P-45 and P-46 are highlighted, which also present a practically smooth pericarp and a blunt or sunken apex after ripening. Hence, they are located mostly on the left of the graph. From G2, and in relation to the second component, varieties P-37 and P-51 and the ungrouped P-49 are located in the lower part of the PCA plot (Figure 2) for their strongest pedicel persistence with fruit in addition to other minor pondered traits such as the marked anthocyanin intensity in their stem nodes in P-37 and P-49, but to a lesser extent in P-51. Complementary information is shown in Figure S5.



*** Figure 8:** Similarities among the pepper fruits belonging to the 17 evaluated pepper landraces based on the traits used for phenotyping represented in the two first components (first component, x-axis; second component, y-axis) of the principal components analysis (21.62% and 12.73% of total variation, respectively). Groups, arranged mainly according to fruit morphology (G1 = thin and elongated, G2 = thick and robust), are represented and rounded in plots G1 (green) and G2 (blue). Ungrouped P-49 (pink) is also expressed in the figure. Light blue arrows represent the eight strongest traits that contribute the most to total diversity among landraces, extracted from a previous feature plot performed with all the traits in the assay (data not shown).

3.6. Correlation among the Selected Quantitative Traits

In order to estimate the contribution and relation between the most important quantitative traits, a correlation analysis was carried out and illustrated by a correlation heat map (Figure 9). The pairwise coefficients are shown in Table S5. The most representative positive correlations were detected for leaf length vs. width, fruit width vs. pericarp thickness, and fruit width vs. fruit fresh weight. These significant pairwise coefficients can be grouped mainly into three kinds of combinations: (1) related to plant size and both leaf dimension and flower/fruit number; (2) fruit width and leaf size; (3) pericarp thickness and fruit fresh and dry matter. The strongest negative relations appeared in three combinations of traits: number of fruit per plant, in particular, vs. fruit width, vs. pericarp thickness, and vs. fruit dry weight



*** Figure 9:** Correlation network discerning the relation among 13 quantitative traits. Correlations between traits are illustrated using different gradient colors of blue and red to discern positive and negative correlations, respectively. The color intensity of the lines connecting traits explains the correlation robustness while Pl: plant, S: stem, L: leaf, Fl: flower, Fr: fruit, FW: fresh weight, DW: dry weight.

4. DISCUSSION

The phenotyping or morpho-characterization of landraces with more descriptors facilitates the identification of discriminatory traits or combinations of traits among accessions [29]. This objective is very important because it determines each cultivar's plant architecture or life cycle by contributing both the agronomic value of landraces and breeding practices; it is an efficient tool for estimating genetic diversity among genotypes because it illustrates divergence [30]. In our study, 20 qualitative and 45 quantitative traits were measured in the vegetative organs, flowers, and both immature and mature fruit of pepper plants. This provided a broader overview (65 data) than similar studies carried out on this crop [11,30–35].

PCA is a key tool for determining the most remarkable traits for landrace characterization in cultivated species, such as pepper [33,34,36–39], sweet potato [40], eggplant [23,41], and tomato [28,42,43] and in ornamental plants such as spider plants [44]. According to our results, when subjecting the phenotypic data of the 17 pepper landraces to PCA analysis, 15 principal components were established and corresponded to 98.92% total variation, but none explained more than 21.82% of diversity among landraces. From this, we inferred wide diversity among accessions even though landraces belonged to the same geographical zone (Valencian Community). Bianchi et al. [30] suggested that accessions from the same regions could not be grouped based on geographic origin. The same conclusion was drawn by Cardoso et al. [35], Baba et al. [45], Moreira et al. [46], and Lahbib et al. [47]. The extended variability in our 17 landraces shown by PCA was associated with different causes. (a) To some extent, ***Capsicum sp***. is considered an autogamous plant. Hence, its reproductive behavior is fairly variable compared to other species, although a certain amount of cross-pollination can occur due to high temperatures, wind, and the presence of insects. Moreover, but no less importantly, (b) plants are transported between regions by human activity, which should not be underestimated, as pointed out by Bozokalfa et al. [11]. This would explain why a correlation is lacking between the geographic and genetic distances found by Finger et al. [48] in peppers. The same conclusion applies to other important cultivated crops like melon [49], lentil [50], sunflower [51], quinoa [52], and garlic [53].

The main principal component to explain 21.8% total variability correlates mostly with fruit descriptors because they are the main differential traits between landraces. The separation of accessions associated with fruit traits is a common practice among the landraces destined for the food market. As consumers accept different pepper sizes, ***Capsicum sp.*** breeding studies have focused on variability characterization for fruit-related traits when selecting promising accessions [34,35,45,47]. A similar conclusion has been reached with other fruit crops, such as eggplant [54,55] and tomato [56], and suggest the key role of morphological variation in this organ during species' domestication process [57]. In particular, the traits related to fruit size, fresh weight, sinuosity, and pericarp thickness were highlighted in the first PCA component, and two groups (G1 and G2) were clearly differentiated. This proved the consistency of the analysis with varietal type, and it also means that genotypes with similar fruit characteristics are generally clustered together. Thus, the fruit from the G1 landraces (left side of the plot) were elongated and light, with marked sinuosity, and had the thinnest pericarps. G2 fruit (right side) were shorter and heavier and had thicker pericarps in both maturity states. Differences with the ungrouped P-49 fruits were even more marked because this landrace presented the shortest fruits with the thickest pericarps, as well as heavy

fruit, at least when immature. A wide variation in fruit diameter and length has also been reported in other studies performed with pepper [30,34,58], and the characteristic that most contributes to genetic divergence is fruit length for [59] and fruit size [35]. Similarly, do Rego et al. [31] reported that fruit length and fruit width are the most important traits for phenotypic divergence (32.3% and 20.6% relative importance, respectively) from a list of 14 analyzed characters. The most discriminating traits for Tembe et al. [33] are fruit shape, width, thickness, and weight, in addition to some quality parameters. For Wasonga et al. [34], a hierarchical cluster analysis grouped the evaluated accessions into eight distinct clusters based on fruit shape, size, surface, and color. According to Bento [60], by means of fruit size, the most appropriate way to use accessions can be determined, as small pepper fruit are mostly employed by the processing industry, while large uniform peppers with good texture or firm fruit are preferably eaten fresh. Regarding pericarp thickness, a wide range (0.04–13.0 mm) was reported by do Rego et al. [31] in 69 accessions of the ***Capsicum genus.*** Lower values for pericarp thickness (1.38–3.08 mm) and apparent less variability were described by Bianchi et al. [30], but that study was carried on 55 accessions from the ***C. chinense*** Jacq species, which are notably smaller than the ***C. annuum*** species. For Tsoney et al. [33], most of the variation in Bulgarian pepper cultivars was manifested by fruit width, pericarp thickness, and fruit weight. These traits are the most discriminative found among the pepper accessions (***C. annuum*** L.) used to study genetic and phenotypic landrace diversity in NW Spain [34] and Tunisia [47].

From the correlation analysis, pericarp thickness correlated positively with both fresh weight and dry matter content. This was the consequence of including fruit from both maturity states in the analysis. As red fruit had thicker walls than green ones, which was especially relevant in landraces G2 and P-49, their water content lowered during the natural ripening process, which increased the dry biomass percentage. The increment in DW biomass during the ripening process has been previously reported [61]. Similar results with a positive correlation between FW and thickness have been obtained by Lannes et al. [62] and Rego et al. [31], although they evaluated only one (ripe) fruit type, as well as a different species (***C. chinense and C. baccatum***, respectively). Moreover, thicker fruits also presented a higher placenta proportion, especially the mature ones, which was the case of some landraces from G2 (P-45, P-47, P-70) and the ungrouped P-49 variety (Figure S1). Both traits have been related to high soluble solid content levels and optimal qualities for the dehydration industry [62]. Placenta size has been related to the accumulation of higher levels of capsaicinoids [47] as they are preferentially synthesized in this tissue [63]. Pericarp thickness is favored by the maturity process, which increases the degree of resistance to pathogens and transportation damage during the postharvest [36,48] to allow longer commercialization periods [64].

Adequate pedicel length is always a desirable trait because it facilities harvest management [32] or is attention-grabbing for leaves to be ornamentally employed [65]. In our study, this trait was not a limiting factor for fruit picking because pedicel length in most landraces ranged from 3.5 to 6.6 cm (Tables 4 and 5), and the minimum for this trait has been determined as 1.5 cm [66]. The interest of our data also lies in dependence on the maturity state as red fruit had shorter pedicels than green ones in all the studied groups/landraces (22.4%, 13.5%, and 18.5% reduction in the G1, G2, and P-49 means, respectively). The negative relation observed between pedicel length and the DW percentage is associated with water loss during maturity. Similarly, some authors describe xylem occlusion in pedicels and the disruption of fruit-to-plant junctions during fruit ripening as a mechanism that leads to fruit hydraulic isolation from the rest of the plant [67,68]. Trifilò et al. [69] determined that the hydraulic architecture in

the fruiting phase of hot pepper plants is clearly addressed to favor water supply to growing fruit. Another correlation was found between pedicel length and fruit size because the longer the pedicels, the longer and narrower the fruit, and, in turn, the capacity to produce fruit was greater. One clear example was the landraces from G1. In contrast, the G2 landraces had fewer fruit plant-1 in relation to their high pericarp thickness values, whose mean value was even more marked upon ripening (53.6% and 67.0% higher than G1 in the green and the red fruit, respectively). The inverse relation between the number of fruit and their weight, which was positive with wall thickness, has also been described by Osschiuto et al. [36] and Lannes et al. [62]. A similar positive correlation has been found by Lahbib et al. [47] between fruit wall thickness vs. fruit width and fruit length vs. fruit plant-1. This means that the strong correlations (significance level $p < 0.001$) between the combination of several of these traits (fruit length vs. fruit width, fruit width vs. fruit plant-1, pedicel length vs. fruit DW, pericarp thickness vs. fruit plant-1) suggest that common or linked genes related to fruit size, pericarp thickness, and pedicel length control these traits, as previously reported [70]. According to do Rego [31], improving fruit wall thickness indirectly improves DW.

Regarding number of locules per fruit (Tables 4 and 5), the mean value in the elongated fruit (G1) was lower than in the G2 varieties (2.9 and 3.5, respectively). The relation of this trait to fruit shape has been previously reported by Bozokalfa et al. [11].

Finally, fruit persistence on pedicel is an important agronomic trait for discriminating the final use of an accession. Searching for an accession with moderately-low persistent fruit that do not drop easily due to wind and rain but drop easily at harvest is a desirable trait when harvesting. This is the tendency in most of the G1 and G2 landraces, which increases with maturity (Figure 8). According to Poulos [71], with pepper breeding for fresh markets, special attention is paid to improvements for easy fruit detachment, plus other qualities (concentrating fruit set and ripening and short crop duration). In contrast, the P-49 landrace displayed moderate-intense persistence, even in the red fruit, due to its likely industrial use and potential mechanical harvest. This trait is also desirable in landraces for ornamental purposes, such as most ***C. chinense*** varieties [72], to maintain plants' esthetic value for as long as possible.

Vegetative characters are also useful to distinguish between ***Capsicum*** landraces, where plant size is a good selection criterion because it influences the destination of plants [47]. Most landraces present plants taller plants 86 cm and an erect growth habit (Figure 2B), which made us realize the worthlessness of these data in our study. However, both are preferential qualities for fruit growers as crop management benefits (area between plants, harvesting, weed control) are evident, which proves the suitability of the studied local landraces. Our values agree with previous reports carried out in ***C. annuum*** plants to be sent to fresh markets [47]. Although yield per plant in pepper is a highly complex issue that is influenced by several traits, some authors have reported a positive correlation between pepper yield and plant size [73,74]. Genotypes with narrower plant widths allow more individuals per area unit, which promotes the better use of cultivation areas and higher economic yields. The importance of canopy diameter has been reported by do Rego et al. [75] to determine the space between plants and rows. Conversely, accessions with intermediate to prostrate growth habits tend to grow to a shorter height and are, therefore, more appreciated on ornamental plant markets [59,76]. The accessions selected for ornamental use, with a canopy diameter and plant height that are 1.5- to 2-fold larger than the pot they are planted in, are recom-

mended, while larger plants are grown as outdoor crops [77].

Moreover, bigger-sized plants grow larger leaves (in both length and width terms), together with more flowers and fruit per plant. The correlations between leaf and fruit traits (positive with fruit width and wall thickness, negative with fruit length) are also notable. A larger foliar area offers a better photosynthate accumulation in plants, which ultimately produces thicker and wider fruit. This occurs in pepper and other crops [61,78]. In contrast, small leaves are interesting for ornamental peppers to better visualize the flowers and fruit [79] and for maintaining harmony with these plants' required small size [77].

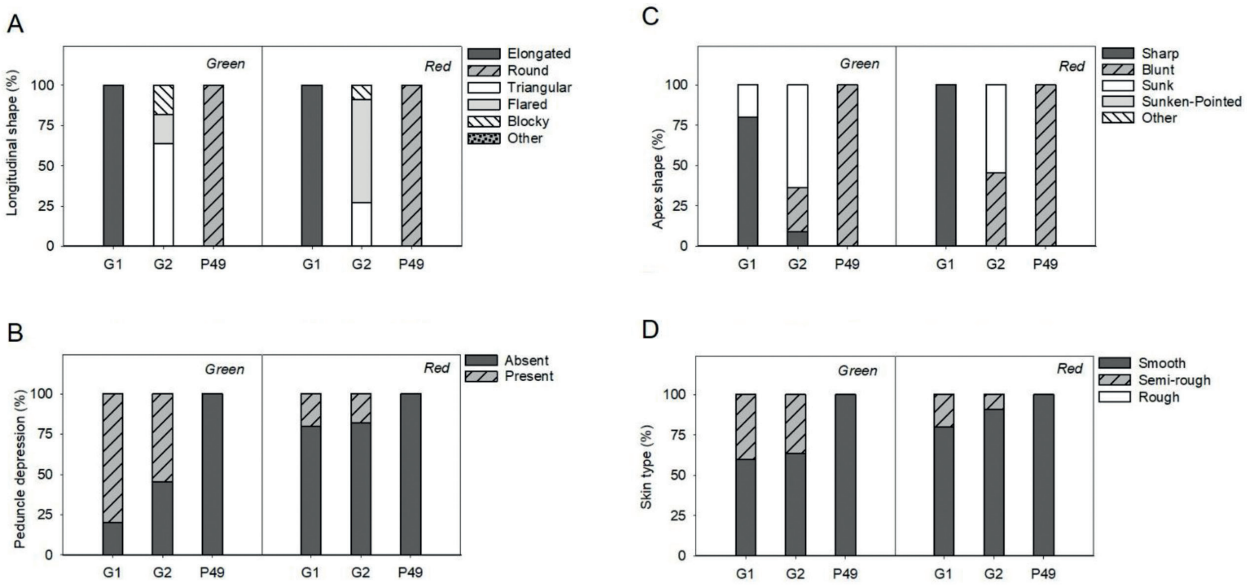
Although stem diameter does not correlate with any of the considered traits, our values exceeded 1.5 cm in all the landraces and even rose to 2.5 cm in P-50 (Table 4). These are good results compared to other studies [32] because the wider the stem, the better the weight support of both the plant and its fruit. For this reason, stem diameter is also considered an important trait for selecting genotypes [79]. Santos Pessoa et al. [32] recommended three genotypes for selection as they presented the widest stem diameter (72% bigger than the mean value) in a study carried out with 16 accessions of ornamental peppers.

In relation to flower traits, most accessions had only one flower per axile (Table 2), which is a typical trait of ***C. annuum***. Although the same value has also been observed in ***C. baccatum*** var. pendulum, this descriptor is considered extremely important for differentiating botanical varieties [29]. In our study, flower traits did not vary much when comparing the different landraces. Independently of fruit shape or market destination, there is a clear trend for erect flowers, serrated and non-pigmented calyxes, and light tones (white or yellow) in filaments, anthers, and the corolla. Lack of flower variability in our landraces contrasts to other authors' findings, who have related variation in flower characters as being fundamental in genetic breeding, given the need to carry out segregating generations and to produce hybrids [80].

5. CONCLUSIONS

Our results showed a high degree of diversity among the selected traditional pepper landraces. The desirable characteristics that may be of interest for culture practices and handling jobs, such as crop harvests, include erect growth habit, dense branching, big leaves, and uniformity and low persistence of fruit. Although landraces are clearly differentiated by their fruit shape (G1 elongated, G2 triangular, square or blocky), some are highlighted for having good attributes for some specific traits. In G1, P-41 produces the longest fruit with thick pericarps and high fresh weight values when the fruit are red. Quite the opposite occurs for the P-72 fruit, which are less elongated but with remarkable wall thickness and fresh weight. From G2, the most productive landrace is P-37, whose big plants produce heavy triangular fruits with high DW values or percentages in both fruit types. The ungrouped P-49 variety can also be promoted for the uniformity of its fruit, thick pericarp, and DW, which all make it an optimal candidate for the industry. Phenotypic description is a useful tool for complementing nutritional and genetic methods to analyze variability in crop diversity studies, identify valuable accessions for breeding programs, develop efficient conservation strategies, and produce detailed agricultural catalogs to facilitate variety selection for every case and need.

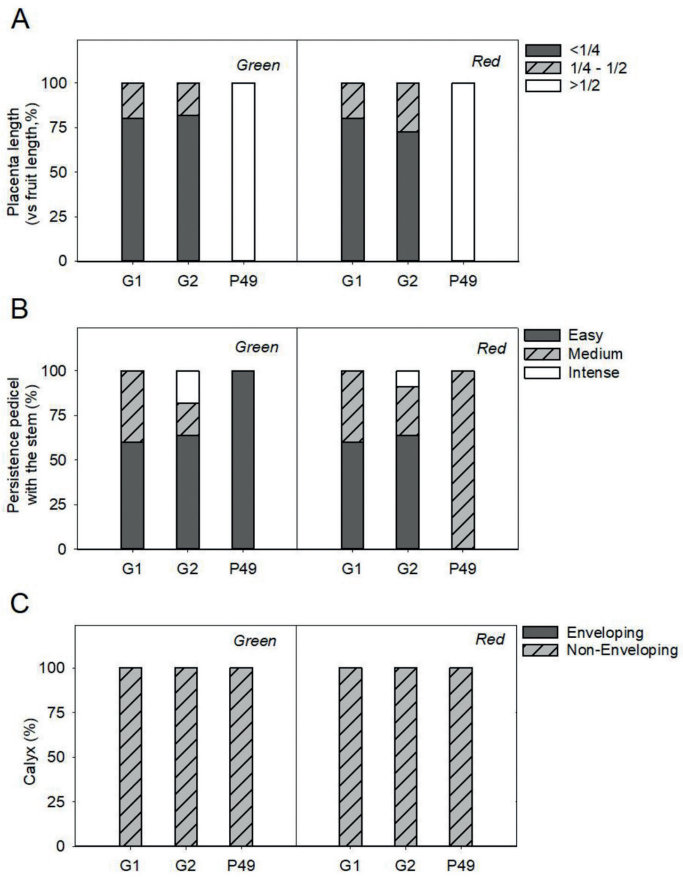
SUPPLEMENTARY



*** Figure S1:** Frequency distribution of selected fruit qualitative trais in 17 pepper landraces in two ripering stages (green and red); (A) Longitudinal shape, (B) Pendule depression, (C) Apex shape, (D) Skin type.
The mean value for each landrace was represented by the most frequent representation of the trait, after classifying the independent fruit samples (n=10) according to the scale (Table S1)

MORPHOLOGIC DESCRIPTOR	*UNIT/SCALE*
QUANTITATIVE	
Stem	
Length until first bifurcation	cm
Diameter	mm
Width	cm
Leaf	
Length	cm
Width	cm
Flower	
Number of flowers per axile	
Fruit (Green/Red)	
Length	cm
Width	cm
Length/width ratio (L/W)	-
Pedicel length	cm
Fruit wall thickness	mm
Locule number	
Fruit fresh weight	g
Fruit dry weight	%
QUALITATIVE	
Plant	
Plant growth habit	3.Postrate 5.Intermediate 7.Erect 9.Other
Height	1.<25cm 2.25-45cm 3.46-65cm 4.66-85cm 5.>86cm
Branch density	3.Scarce 5.Intermediate 7.Dense
Stem	
Shape	1.Cylindrical 2.Angular 3.Flattened
Colour	1.Green 2.Green with purple stripes 3.Other
Anthocyanin colour in the node	1.Green 2.Light purple 3.Purple 4.Dark purple
Leaf	
Shape	1.Deltoid 2.Oval 3. Lanceolate
Colour	1.Yellow 2.Light green 3.Green 4.Dark green 5.Light purple 6.Purple 7.Marbled 8.Other
Flower	
Position	3.Pending 5.Intermediate 7.Erect
Calyx margin	1.Integer 2.Intermediate 3.Serrated 4.Other
Filament colour	1.White 2.Yellow 3.Green 4.Blue 5.Light purple 6.Purple 7.Other
Anther colour	1.White 2.Yellow 3. Pale blue 4.Blue 5.Purple
Stigma insertion	1.Insert 2.At the same level 3.Exserted
Calyx pigmentation	1.Absent 2.Present
Corolla colour	1.White 2.Light yellow 3.Yellow 4.Greenish yellow 5.Purple with white base 6. White with purple base 7.Purple 8.White with purple margin 9.Other
Fruit (Green/Red)	
Attitude	1.Erect 2.Horizontal 3. Inclined
Skin brigthness	1.Weak 2.Medium 3.Intense
Longitudinal shape	1.Elongated 2.Almost round 3.Triangular 4.Flared 5.Blocky 6.Other
Shape at the junction with the pedicel	1.Sharp 2.Obtuse 3.Truncated 4.Chordate 5.Lobulated
Peduncle depression	0.Absent 1.Present
Cross wrinkle	3.Slight 5.Intermediate 7.Very corrugated
Apex shape	1.Sharp 2.Blunt 3.Sunk 4.Sunken and pointed 5.Other
Pericarp sinuosity	1.Absent or very weak 2.Weak 3.Medium 4.Strong 5.Very strong
Skin type	1.Smooth 2.Semi-rough 3.Rough
Placenta length	1.<1/4 fruit length 2.1/4-1/2 fruit length 3.>1/2 fruit length
Persistence pedicel with the fruit	3.Easy 5.Medium 7.Intense
Persistence pedicel with the stem	3.Easy 5.Medium 7.Intense
Calyx	1.Enveloping 2.Non-enveloping
Apical rot	1.Absent 2.Weak 3.Medium 4.Strong
Cracked	0.Absent 3.Weak 5.Medium 7.Strong

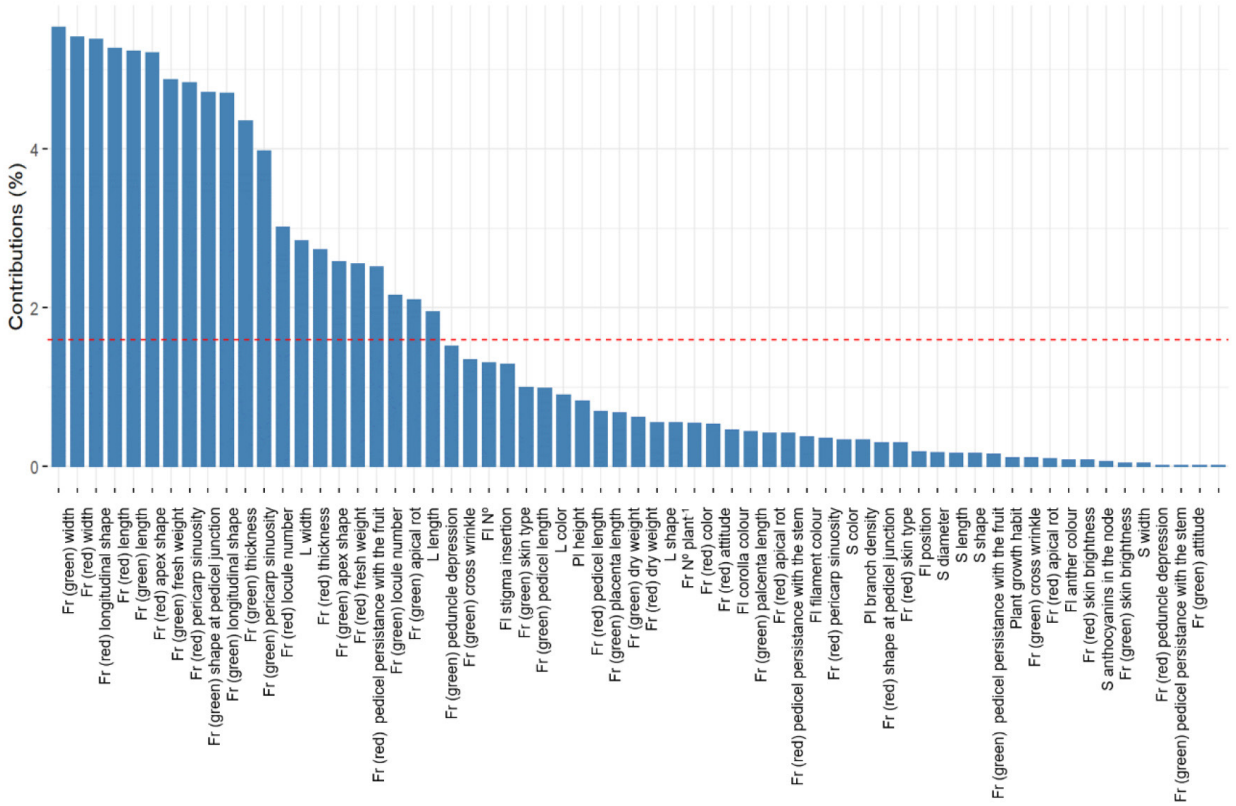
*** Table S1:** Descriptors used for phenotyping according to the International Board for Plant genetic resources descriptors (IBPGR 1995) for pepper.



*** Figure S2:** Frequency distribution of selected fruit qualitative trais in 17 pepper landraces in two ripering stages (green and red); (A) Placenta lenght, (B) Persistence pedicel with the stem, (C) Calyx. The mean value for each landrace was repre sented by the most frequent representation of the trait, after classifying the independent fruit samples (n=10) according to the scale (Table S1).

	G1					G2					P-49				
	Mean	Range	CV%	F-ratio		**Mean**	Range	CV%	F-ratio		**Mean**	Range	CV%	F-ratio	
Vegetative traits															
Stem length until first bifurcation (cm)	**27,7**	15-39	21,6	18.6***	b	**29,1**	10-46	22,4	16.6***	b	**33,9**	35-39			a
Stem diameter (mm)	**15,2**	11.8-19.5	10,9	1,0		**16,2**	11.6-33	22,2	19.3***		**15,7**	14.3-17.7			
Plant width (cm)	**65,5**	45-96	14,5	1.86*		**63,0**	34-87	18,9	4.2***		**59,7**	57-61			
Leaf length (cm)	**18,2**	12.5-25.8	13,5	3.22*	b	**20,2**	13.5-27	13,8	7.0***	a	**21,0**	20.1-22.7			a
Leaf width (cm)	**9,8**	13.3-5.8	13,8	0,9	b	**11,5**	8.5-16.9	15,4	12.8***	a	**12,4**	11.8-13.5			a
Flowes per axile	**1,00**	-	0,0	1,0	b	**1,32**	1-3	39,1	12.3***	a	**1,00**	-			b
Fruit traits (Green)															
Length (cm)	**17,9**	9.2-28.3	20,2	2,1	a	**11,6**	5.2-17.3	24,0	30.2***	b	**6,1**	5.5-6.7			c
Width (cm)	**3,6**	1.12-6.18	31,1	20.78***	b	**6,8**	3.9-10.4	18,9	15.7***	a	**7,0**	6.6-7.9			a
Length/width ratio	**5,4**	3-9.29	30,2	15.71***	a	**1,8**	0.8-3.8	34,2	25***	b	**0,9**	0.8-1.0			c
Pedicel length (cm)	**4,9**	2.8-7.3	21,8	22.51***	a	**4,3**	0.9-6.5	25,5	17.8***	b	**4,0**	2.8-4.8			b
Fruit wall thickness (mm)	**3,0**	1.18-4.9	27,9	19.5***	c	**4,7**	3.0-6.9	17,1	3.9***	b	**7,1**	6.1-8.8			a
Locule number	**2,9**	2-5	22,9	4.71**	b	**3,5**	2-5	15,9	6.3***	a	**2,8**	2-4			b
Fresh weight (g)	**84,1**	58.93-110.4	16,5	6.11**	b	**142,0**	87.3-203.2	16,5	3.3**	a	**124,7**	105.7-141.2			a
Dry weight (%)	**6,8**	5.64-8.72	14,1	59.91***	a	**5,9**	5.2-7.5	7,9	6.4***	b	**6,3**	5.6-6.9			ab
Fruit traits (Red)															
Length (cm)	**17,9**	11-24.6	23,3	19.59***	a	**10,9**	6.5-17.7	17,8	3.9***	b	**5,7**	4.4-6.9			c
Width (cm)	**3,5**	2-6.1	26,5	7.26***	b	**6,8**	3.8-9.9	21,8	11.1***	a	**6,7**	6.2-7.4			a
Length/width ratio	**5,2**	2.9-6.93	19,3	2.71*	a	**1,6**	0.9-2.9	26,9	14.1***	b	**0,9**	0.7-1.2			c
Pedicel length (cm)	**3,9**	2.5-5.9	21,6	20.44***		**3,6**	2-5.6	20,4	4.9***		**3,2**	2.6-4.1			
Fruit wall thickness (mm)	**3,0**	1.89-4.1	18,1	14.7**	c	**5,2**	2.5-11.1	37,3	56.1***	b	**8,2**	7.3-9.4			a
Locule number	**3,0**	2-5	23,3	3.81*	b	**3,6**	3-4	14,1	0,7	a	**3,3**	3-4			b
Fresh weight (g)	**82,2**	41.45-116.7	26,1	5.51**	b	**113,6**	73.5-153.6	16,6	2.27*	a	**86,1**	80.7-91.5			b
Dry weight (%)	**10,6**	8.13-15.4	19,4	11.54***	a	**9,5**	6.0-13	9,3	3.2**	b	**10,9**	10.1-12.8			ab

*** Table S2:** Descriptive statistics and analysis of variance (ANOVA) of quantitative traits for conventional morphologic descriptors in vegetative organs (plant, leaf and flower) and fruits (green and red) of 17 local landraces of pepper cultivated in Spain. Statistics were performed by the formed groups based on fruit shape; G1= elengated, G2= triangular, square or blocky. Data belonging to outlier (P-49) is also shown. In each group, values represent the mean, range, coefficient of variation (CV, %), F-ratio and significance (***,**,* indicate significance at < 0.001, p< 0.01, p<0.05), for the conventional morphological descriptors studied (n=8 for plant, leaf and flower traits and n=10 for fruit traits). Different letters in a row indicate significant differences at < 0.05 (LSD test)



*** Figure S3:** Variable contribution (%) of qualitative and cuantitative traits used for phenotyping the collection of 17 pepper landraces cultivated in Spain, in relation to the first PC. Pl: Plant, S: Stem, L: Leaf. FL flower, Fruit, FW: Fresh Weight, DW: Dry Weight

Table S3. PCA Eigenvalue, contribution of each PC % Variance, and % Cumulative Variance

PC	Eigenvalue	Variance (%)	Cumulative Variance (%)
1	13,62	21,62	21,62
2	8,02	12,73	34,35
3	6,08	9,65	44,00
4	5,49	8,71	52,71
5	4,19	6,65	59,36
6	3,90	6,19	65,54
7	3,72	5,90	71,44
8	3,22	5,11	76,55
9	2,79	4,43	80,98
10	2,60	4,13	85,11
11	2,37	3,75	88,87
12	2,19	3,47	92,34
13	1,68	2,67	95,00
14	1,32	2,09	97,10
15	1,10	1,75	98,85
16	0,73	1,15	100
17	1,32E-15	0	100
18	1,11E-15	0	100
19	9,77E-16	0	100
20	9,47E-16	0	100
21	8,49E-16	0	100
22	8,01E-16	0	100
23	7,36E-16	0	100
24	6,53E-16	0	100
25	5,87E-16	0	100
26	5,65E-16	0	100
27	4,74E-16	0	100
28	4,50E-16	0	100
29	4,26E-16	0	100
30	3,04E-16	0	100
31	2,59E-16	0	100
32	2,14E-16	0	100
33	2,04E-16	0	100
34	1,61E-16	0	100
35	1,40E-16	0	100
36	1,05E-16	0	100
37	9,00E-17	0	100
38	5,65E-17	0	100
39	0	0	100
40	0	0	100

***Table S3:** PCA Eigenvalue, contribution of each PC% Variance, and % Cumulative Variance

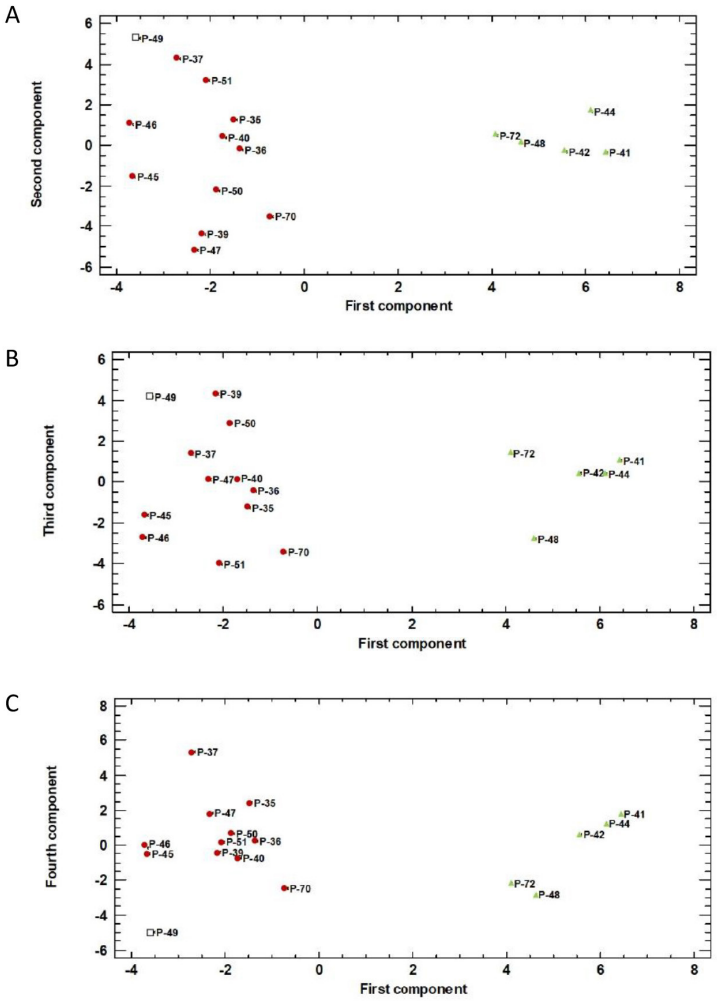


***Figure S4:** Similarities among the pepper fruits belonging to the 17 evaluated pepper lamdraces based on the traits used for phenotyping represented in: A) the two first components (first component, x-axis; second component, y-axix) of the principal components analysis (21.62% and 12.73% of total variation, respectively); B) the first and the third components (first components, x-axis; third component, y-axis) of the principal componets analysis (21.62% and 9.65% of total variation, respevtively); C) the first and fourth components (first component, x-axis; fourth component, y-axis) of the principal component analysis (21.62% and 8.71% of total variation, respectively). Groups arranged based mainly on fruit morphology (G1=thin and elogated, G2= thick and robust), are represented in plots G1 (▲) and G2 (●). Non-grouped P-49 (□) is also expressed in the figure.

	First PC	Second PC	Third PC	Fourth PC
Pl Growth habit		0,172	**-0,194**	
Pl Branch density				**0,26**
Pl Height				
S colour				0,213
S anthocyanins in the node		**0,295**		
S shape				
S length			0,158	
S diameter			0,15	
S width		0,207	**-0,221**	
L colour				
L shape		**0,226**	-0,162	
L length				
L width	-0,168			
Fl per axile				0,166
Fl position		**-0,197**	**0,217**	
Fl corolla colour		**-0,203**		
Fl anther colour		-0,18		0,171
Fl filament colour				**0,224**
Fl stigma insertion		0,153		
Fr (green) attitude		0,148	**0,263**	
Fr (green) skin brightness				**0,296**
Fr (green) longitudinal shape	-0,217		-0,167	
Fr (green) shape at pedicel junction	-0,217			
Fr (green) peduncle depression				
Fr (green) apex shape	-0,16		-0,196	
Fr (green) cross wrinkle		**-0,267**		
Fr (green) pericarp sinuosity	**0,199**			
Fr (green) skin type				**0,22**
Fr (green) pedicel persistence with the fruit				-0,172
Fr (green) pedicel persistence with the stem			**-0,214**	
Fr (green) placenta length		0,198	**0,196**	
Fr (green) apical rot				
Fr (green) cracked				
Fr (green) length	**0,229**			
Fr (green) width	**-0,235**			
Fr (green) pedicel length				
Fr (green) thickness	-0,208			-0,152
Fr (green) locule number			-0,17	
Fr (green) fresh weight	**-0,221**			
Fr (green) dry weight			**0,246**	
Fr (red) attitude		-0,162		**-0,248**
Fr (red) colour				**-0,253**
Fr (red) skin brightness		0,187		
Fr (red) longitudinal shape	**-0,232**			
Fr (red) shape at pedicel junction				-0,194
Fr (red) peduncle depression		-0,181		
Fr (red) apex shape	**-0,228**			
Fr (red) cross wrinkle				
Fr (red) pericarp sinuosity	0,22			
Fr (red) skin type			-0,179	
Fr (red) pedicel persistence with the fruit	-0,158	**0,225**		
Fr (red) pedicel persistence with the stem		0,186		**0,267**
Fr (red) placenta length			**0,218**	
Fr (red) apical rot			0,134	**-0,235**
Fr (red) cracked				
Fr (red) length	0,229			
Fr (red) width	**-0,232**			
Fr (red) pedicel length		-0,175		
Fr (red) thickness	-0,165			
Fr (red) locule number	-0,173			
Fr (red) fresh weight	-0,159			
Fr (red) dry weight			**0,312**	
Fr N° plant^{-1}		0,182		
Eigenvalue	13,62	8,01	6,08	5,49
Variance explained (%)	21,62	12,73	9,65	8,71
Cumulative variance explained (%)	21,62	34,35	44	52,71

*** Table S4:** Correlation coefficients for each morphological trait for the four first principal components, eigenvalue, and relative and cumulative proportion of the total variance explained by these components in the collection of the 17 local landraces of pepper cultivated in Spain. This table shows the principal component equations, where the values of the variables in the equation have been standardized by subtracting their mean and dividing by their standard deviations. Only the correlations with absolute value≥0.150 are listed. Pl: Plant, S: Stem, L: Leaf, Fl: Flower, Fr: Fruit.

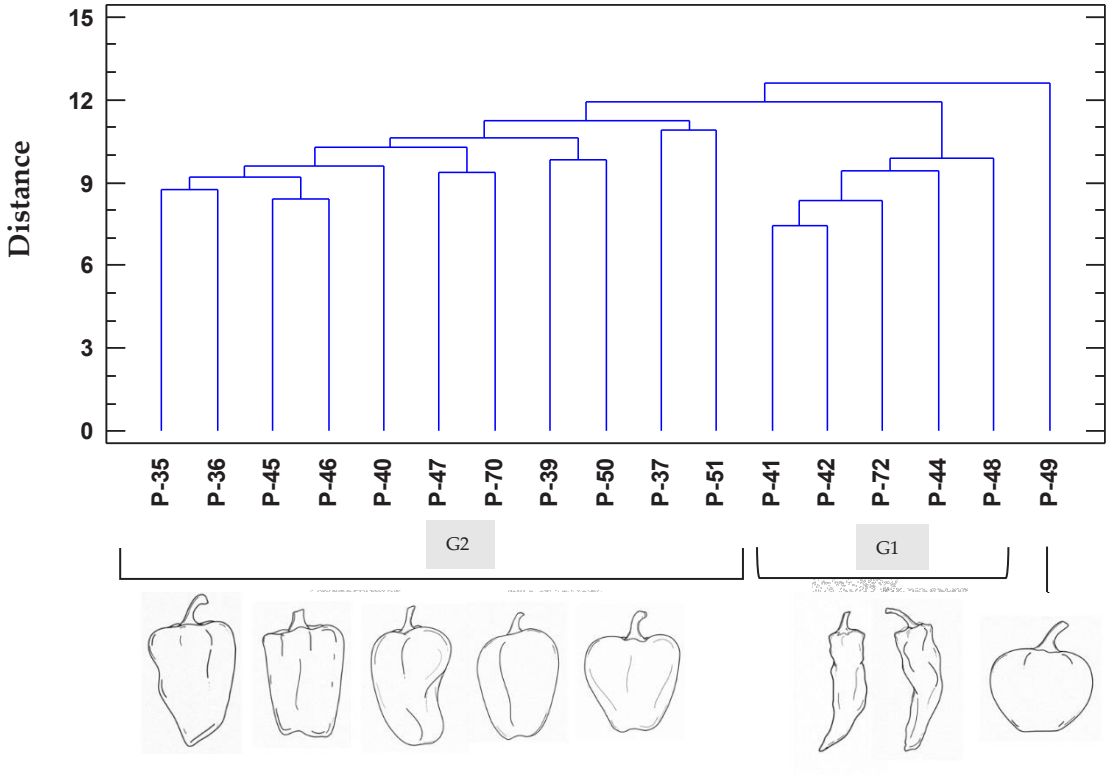


***Figure S5:** Clustering analysis among 17 pepper landraces construced based on the traits used for the phenotypic characterization of the varieties, using Group mean method for clustering. Formed groups; G1= elongated, G2=triangular, square or blocky. Euclidean Distance= Coefficient of similarity

TRAITS	Pl width	S length	S diameter	L length	L width	Fl N° inflorescence^{-1}	Fr length	Fr width	Fr Pedicel length	Fr Pericarp Thickness	Fr FW	Fr DW (%)	Fr N° plant^{-1}
Pl width		-0,1172	-0,1072	-0,0619	0,0089	**0.2624****	-0,0476	-0,0162	0,0654	-0,0295	-0,1261	-0,1801	**0.2804***
S length			0,0544	**0.2855*****	**0.4037*****	-0,0806	-0,1284	0,0693	-0,0365	**0.1713***	0,0031	0,1275	-0,2623
S diameter				-0,0659	-0,0402	0,0609	0,0018	0,0506	-0,0387	0,1315	0,0345	0,0205	-0,0849
L length					**0.7628*****	0,0157	**-0.2548****	**0.2616****	-0,16	**0.2121***	0,1723	-0,0333	-0,1235
L width						0,1494	**-0.3598*****	**0.3287*****	-0,1036	**0.2903*****	**0.2499***	-0,0402	-0,2715
Fl N° inflorescence^{-1}							**-0.2032***	**0.319*****	-0,0294	**0.1781***	0,1397	-0,0541	-0,1101
Fr length								**-0.4924*****	**0.1509***	**-0.3505*****	**-0.3998*****	**-0.2275***	**0.4058****
Fr width									**-0.1657****	**0.5734*****	**0.6049*****	0,0374	**-0.5193*****
Fr Pedicel length										**-0.1881****	0,0013	**-0.3844*****	**0.3602***
Fr Pericarp Thickness											**0.3117*****	**0.3667*****	**-0.6457*****
Fr FW												**-0.2364****	**-0.5323*****
Fr DW (%)													**-0.2986***
Fr N° plant^{-1}													

*** Table S5:** Linear correlation coefficient (r) and its significance between quantitative traits used in phenotyping in the collection of 17 pepper landraces cultivated in Spain. ***, **, * indicate significant at P<0.001, P<0.01 and P<0.05 values for r. Pl: Plant, S: Stem, L: Leaf, Fl: Flower, Fr: Fruit, FW: Fresh Weight, DW: Dry Weight.

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CHAPTER 04

BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF VALENCIAN PEPPER LANDRACES

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Abstract
Sweet pepper is one of the most important economic fruits with nutritional attributes. In this sense, the nutraceutical value of consumed products is a major concern nowadays so the content of some bioactive compounds and antioxidants (phenols, ascorbic acid, lycopene, carotenoids, chlorophylls, and antioxidant activity) was monitored in 18 sweet pepper landraces at two maturity stages (green and red). All the traits except chlorophylls significantly increased in red fruits (between 1.5- and 2.3-fold for phenols, ascorbic acid, and2-2-diphenyl-1-picrylhydrazyl (DPPH) inhibition activity, 4.8-fold for carotenoid and 27.4-fold for lycopene content), which suggests that ripening is key for obtaining desired fruit quality. Among landraces, P-44 in green fruits is highlighted for its content in carotenoids, chlorophylls, phenols, and ascorbic acid, and P-46 for its antioxidant capacity and lycopene content. Upon maturity, P-48, P-44, and P-41 presented higher levels of phenols and lycopene, and P-39 of phenols, carotenoid, and DPPH. This work reflects a wide variability in the 18 pepper landraces at bioactive compounds concentration and in relation to fruit ripeness. The importance of traditional landraces in terms of organoleptic properties is emphasized as they are the main source of agricultural biodiversity today and could be helpful for breeders to develop new functional pepper varieties.

Keywords
antioxidant activity; ascorbic acid; bioactive compound; carotenoids; landrace; lycopene; phenols; pepper.

1. INTRODUCTION

The key role of diet in preventing illnesses has long been recognized, and people have become more careful with the food they choose to consume by seeking foods with higher nutritional values [1]. This is why many recent research works have focused on the determination and quantification of important bioactive compounds present in plant and food materials.

Traditional varieties or landraces are those that have been differentiated by farmers by means of a historic selection process. Therefore, they represent great genetic heritage as a source of agricultural biodiversity [2], which is a key element for ensuring food quality. In fact, consumer demand for vegetable landraces is increasing worldwide, mainly for their sensory values. Additionally, local varieties are better adapted to specific agroclimatic conditions and are, thus, especially recommended for new kinds of agriculture based on sustainable and low inputs, such as organic production [3,4]. The characterization and use of landraces offer a new chance to improve crop organoleptic quality. This opportunity is applicable to those areas in which genetic erosion resulted in a dramatic loss of biodiversity [5,6] that has derived from agrarian mechanization and prioritization of size uniformity and external fruit aspect when choosing profitable crops for the food industry [7]. However, an opposite trend has recently been set by consumers, who have voiced concern about the organoleptic quality and nutritional value of local products [8,9]. This has led to an increase in the number of research studies that focus on bioactive compound extraction from fruit and vegetables, while studying their impact on the human body [10].
Pepper plants ***(Capsicum spp.)*** belong to the Solanaceae crop family, formed by vegetables of remarkable agro-economic importance worldwide [11], among other species that make this family one of the most important ones for human diet for their multiple applications [12]. Of the five domesticated ***Capsicum species,*** the most important from the agronomic point of view is the sweet pepper ***Capsicum annuum*** L., which has wide phenotypic variability, as well as offering countless cooking applications [13]. Pepper is the second most consumed vegetable worldwide, hence its considerable agronomic and economic importance (1.99 million cultivated hectares (ha) and 38 million tonnes production, 2019) [14]. Furthermore, its nutritional value is important because it is rich in ascorbate (vitamin C), β-carotene (provitamin A), calcium, α-tocopherol (vitamin E), thiamine (B1), riboflavin (B2), niacin (B3), and antioxidants like carotenoids and phenolic acids [15].

The study was carried out in 18 local pepper landraces from the Valencian Community (Spain) that represent a wide phenotype variety. Plant resources were provided by the Valencian germplasm bank (GB) from Valencian Institute for the Conservation and Improvement of Agrobiodiversity (COMAV, Valencia, Spain) and the Valencian Institute for Agrarian Research (IVIA, Valencia, Spain). The aims of this research were: 1) determine the nutritional and nutraceutical characteristics of all the pepper landraces, and 2) to value the most promising materials from a nutritional point of view in order to know the health benefits deriving from their use by considering two maturity stages. This work can contribute to revalue traditional landraces by emphasizing their nutritional values such as added value, and additionally, to enhance certain endangered traditional varieties and promote their use and conservation.

2. RESULTS

2.1. Nutraceutical Compounds and Antioxidant Capacity

2.1.1 Phenols

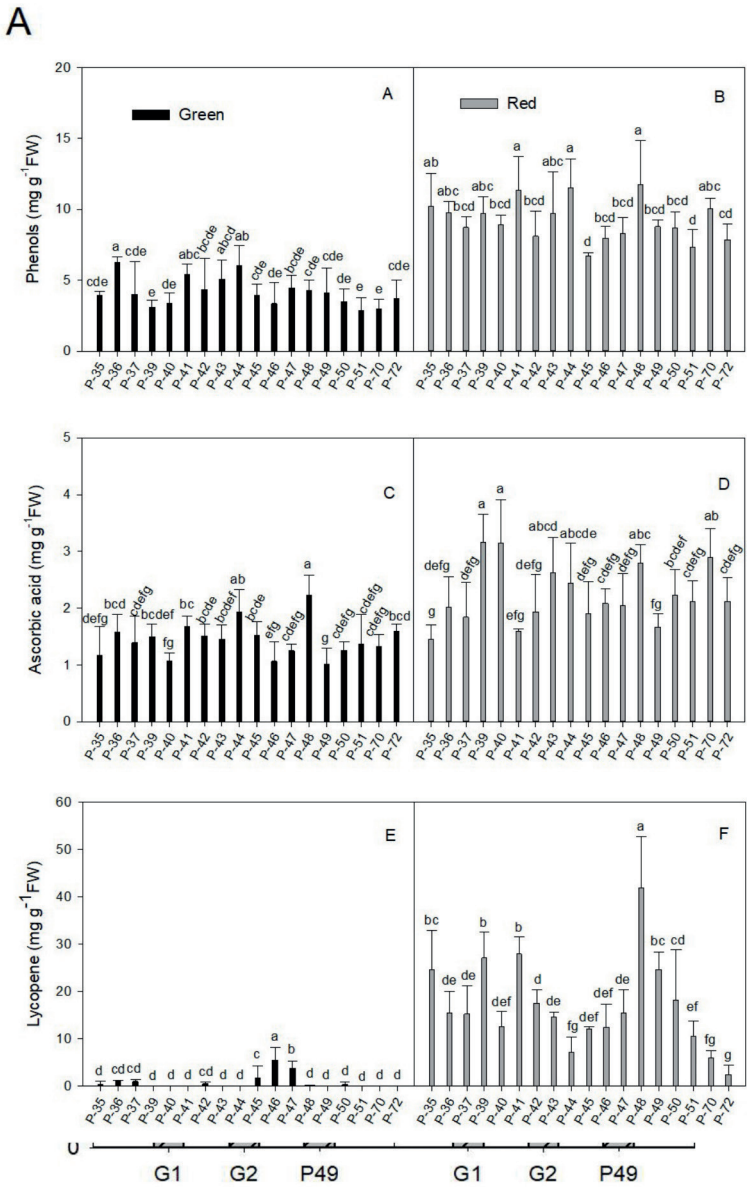
Phenols are phytochemical compounds of interest in pepper fruit for their ability to scavenge free radicals. Table 1 and Figure 1 show the concentration of phenols in green and red fruits of the 18 pepper landraces. Phenol concentration was significantly higher in the red than in the green fruits (mean values of 9.20 and 4.17 mg g-1 FW, respectively; Table 1). The mean values of phenols for the different landraces in green fruits ranged between 1.83 and 7.24 mg g-1 FW (Table 1). Some landraces (P-36, P-41, P-43, and P-44) stood out for their high phenol concentration (ranging from 5.08 to 6.25 mg g-1 FW) (Figure 1A). No differences were found for this trait in the other landraces, whose average phenol concentration was 3.72 ± 0.52 mg g-1 FW.

The individual phenol concentration data in the red fruits presented a wider range than in green fruits, while the coefficient of variation in the former was slightly lower than in the latter (21.5% and 34.7%, respectively).

Phytochemicals Concentration	Unit/Scale	Mean	Range	CV (%)	F-Ratio
Green Fruit					
Phenols	$mg\ g^{-1}$ FW	4.17 ± 1.43	1.83–7.24***	34.32	2.59
Ascorbic acid	$\mu g\ g^{-1}$ FW	1.44 ± 0.40	0.60–2.47***	27.93	3.65
Lycopene	$mg\ g^{-1}$ FW	0.62 ± 1.4	0–8.25**	225.06	2.46
Carotenoid concentration	$\mu g\ g^{-1}$ FW	6.35 ± 2.15	2.64–13.11*	33.87	2.12
Chlorophyll a + b	$\mu g\ g^{-1}$ FW	35.07 ± 10.97	14.85–62.82**	31.29	2.85
Antioxidant capacity	% FW	36.57 ± 17.88	6.12–77.84***	48.91	8.31
Red Fruit					
Phenols	$mg\ g^{-1}$ FW	9.20 ± 1.97	5.66–15.87***	21.45	3.20
Ascorbic acid	$mg\ g^{-1}$ FW	2.24 ± 0.66	1.05–3.94***	29.86	3.73
Lycopene	$mg\ g^{-1}$ FW	16.98 ± 10.11	0.42–49.28***	59.51	15.50
Carotenoid concentration	$\mu g\ g^{-1}$ FW	30.58 ± 14.05	12.17–103.88***	45.95	3.38
Chlorophyll a + b	$\mu g\ g^{-1}$ FW	1.18 ± 1.10	0–5.31	93.18	1.46
Antioxidant capacity	% FW	83.92 ± 12.4	49.68–96.31***	14.77	19.42

Values represent the mean, range, significance (***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively), coefficient of variation (CV %), and F-ratio for the quality traits studied.

*** Table 1.** Variation parameters for quality traits in 18 pepper landraces cultivated in Spain.



*** Figure 1.** Total phenols (A and B), total ascorbic acid concentration (C and D), and lycopene concentration (E and F) in the green (A, C, and E) and red (B, D, and F) fruit produced by the 18 pepper landraces. Values are the mean ± SE of four replicates per landrace. Mean is subjected to a one-way ANOVA and different letters indicate significant differences at $p < 0.05$ using the LSD test.

The individual phenol concentration data in the red fruits presented a wider range than in green fruits, while the coefficient of variation in the former was slightly lower than in the latter (21.5% and 34.7%, respectively).

In red fruits, the mean values for the different landraces ranged between 5.66 and 15.87 mg g-1 FW (Table 1). The highest phenol values in mature fruits were recorded in four landraces (P-35, P-41, P-44, P-48) whose concentrations ranged from 10.24 to 11.74 mg g-1 FW (Figure 1B). The lowest phenol values in red fruits were detected in P-45 and P-51 (6.74 and 7.34 mg g-1 FW, respectively).

In addition, although the phenol concentration in all the landraces significantly increased with maturity (2.1-fold on average), some landraces showed more remarkable raises. This occurred with P-35, P-39, P-40, and P-48 (between 2.61- and 3.12-fold) and P-70, which displayed the most marked increase in phenol concentration between red and green fruits throughout the experiment (3.35-fold). In contrast, this rise was only 1.5-fold in landrace P-36 which, despite being the lowest, showed a statistically significant increase between red and green fruits.

2.1.2 Total Ascorbic Acid

Red fruits presented a higher ascorbic acid concentration than the green ones (average of 2.24 and 1.44 mg g-1 FW, respectively; Table 1). Both ripening stages presented a similar coefficient of variation (around 29%). Three landraces (P-40, P-46, P-49) in green fruits had the lowest ascorbic acid concentration, close to 1 mg g-1 FW (Figure 1C) and it doubled in P-44 and P-48 (1.93 and 2.17 mg g-1 FW, respectively). In red fruits (Figure 1D), some landraces (P-39, P-40, P-48, P-70; Figure 1D) stood out for their high vitamin C concentration (between 2.80 and 3.16 mg g-1 FW), whereas three landraces (P-35, P-41, P-49) obtained the lowest values (around 1.57 ± 0.11 mg g-1 FW). When comparing red and green fruits, the rise in the ascorbic acid concentration in mature fruits was statistically significant (more than 1.7-fold) in several landraces (P-39, P-40, P-43, P-46, P-47, P-50, P-70). The highest increase (three-fold) was recorded for P-40.

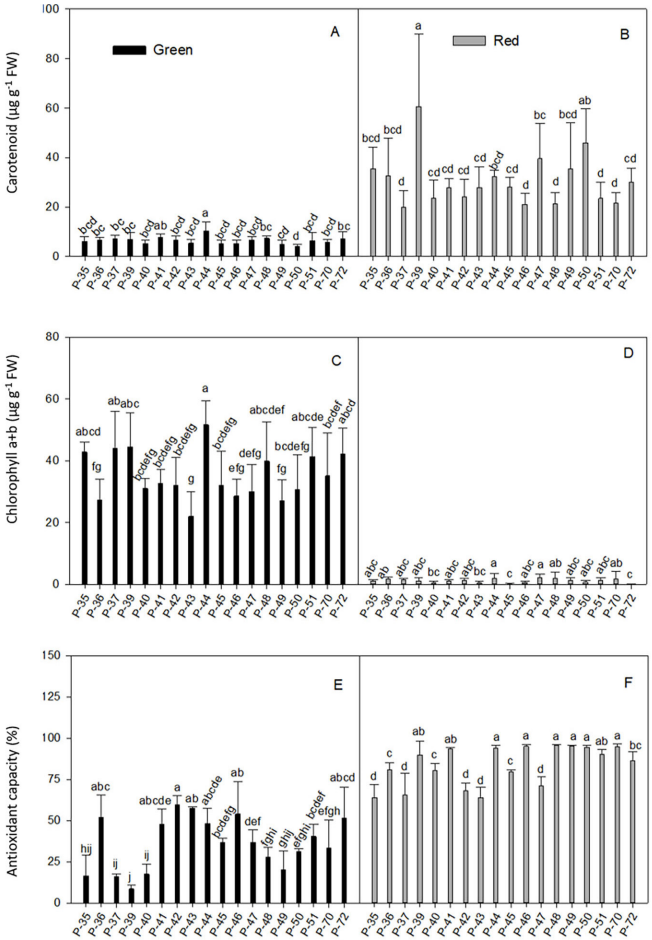
2.1.3 Lycopene

Lycopene concentration was higher in red fruits than in their green counterparts (16.98 and 0.62 mg g-1 FW, respectively; Table 1). Although mature fruits presented a wider range of individual data, the coefficient of variation was 3.8-fold higher in immature fruits (59.5% and 225.1%, respectively).

The extremely low signal recorded in some extracts of the green fruits suggested that lycopene was almost absent (Figure 1E). This was the case of P-39, P-40, P41, P-43, P-44, P-48, P-49, P-51, P-70, and P-72. In contrast, three landraces (P-45, P-46, and P-47) stood out for having a relatively high lycopene concentration in green fruits (1.75, 3.84, and 2.75 mg g-1 FW, respectively). The maturity process improved lycopene content in all the landraces and the average concentration increased by 25.6-fold when comparing red and green fruits (Table 1). In red fruits (Figure 1F), four landraces (P-35, P-39, P-41, P-49) had a lycopene concentration of around 25.0 mg g-1 FW, which rose to 41.83 in P-48, which was the highest value (41.83 mg g-1 FW). P-44, P-70, and P-72 presented the lowest values (between 2.5 and 7.0 mg g-1 FW), which were around three-fold lower than the average for mature fruits.

2.1.4 Carotenoid

In general terms, carotenoid concentration (Figure 2A) was similar in green fruits from different landraces (average 6.35 µg g-1 FW; Table 1). Only P-44 is highlighted for its high value (10.3 µg g-1 FW; Figure 2A), while two varieties (P-49 and P-50) had low levels (4.81 and 3.99 µg g-1 FW, respectively). Once again, maturity significantly increased carotenoid concentration (4.82-fold; Table 1) and despite the variability in the individual data (45.9% of the coefficient of variation), the average value in most landraces ranged from 21.0 to 35.4 µg g-1 FW (Figure 2B). A more marked increase was recorded (8.74-, 7.36- and 11.51-fold) for three varieties (P-39, P-47, and P-50, respectively) when comparing red and green fruits.



*** Figure 2.** Carotenoid (A and B), chlorophyll a + b (C and D), and antioxidant capacity (E and F) in the green (A, C, and E) and red (B, D, and F) fruit produced by the 18 pepper landraces. Values are the mean ± SE of four replicates per landrace. Mean is subjected to a one-way ANOVA and different letters indicate significant differences at $p < 0.05$ using the LSD test.

2.1.5 Total Chlorophyll Concentration

The total Chls concentration was strongly influenced by fruit maturity status (average 35.07 µg g-1 FW in green and 1.18 µg g-1 FW in red fruits; Table 1). In green fruits (Figure 2C), Chl concentration ranged between 40.23 and 44.53 µg g-1 FW in five landraces (P-35, P-37, P-39, P-51, P-72) and rose to 51.65 µg g-1 FW in P-44. Three landraces (P-36, P-46, P-49) had very low values (between 27.1 and 28.6 µg g-1 FW) and P-43 obtained the lowest value (22.05 µg g-1 FW), which was 2.3-fold lower than for P-44.

In red fruits (Figure 2D), two accessions (P-45 and P-72) are highlighted for their very low Chl content (0.15 and 0.08 µg g-1 FW, respectively), which was around 7.9-fold lower than the average value of all red fruits included in the experiment (1.18 µg g-1 FW; Table 1). However, the highest value for the whole experiment (2.05 µg g-1 FW) was for landrace P-44, which was only 1.9-fold higher than the average value.

When comparing the total results between maturity stages, the average Chl concentration (Table 1) in green fruits was 29.7-fold higher than in red ones (35.07 and 1.18 µg g-1 FW, respectively). Despite the low data range in mature fruits, the average value was not statistically significant given the high coefficient of variation (93.2%). Among the landraces, the reduction in the total Chl concentration between green and red fruits was between 95% and 97%, and even reached 99% in three varieties (P-40, P-45, and P-72). The least reductions (around 93%) were observed in P-36 and P-47.

2.1.6 Antioxidant Capacity

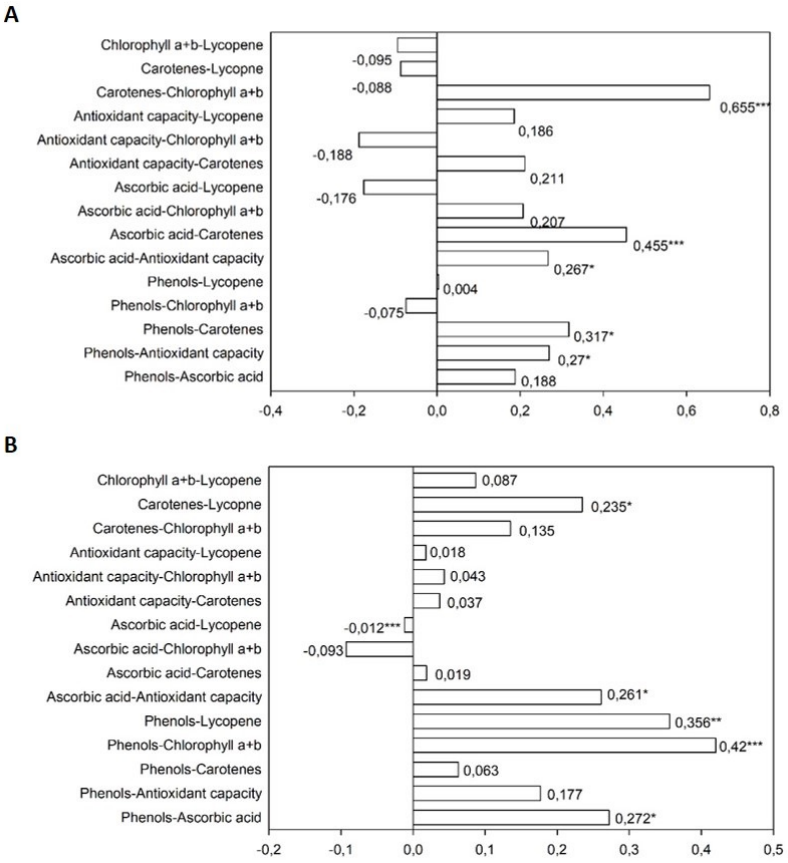
Antioxidant capacity, as determined by the DPPH assay, was significantly higher in red fruits than in green ones (83.92 and 36.57%, respectively; Table 1). However, immature fruits presented more variability, and the DPPH inhibition range was wider than in mature ones. This was reflected by the coefficient of variation, which was three-fold higher in green than in red fruits (48.9 and 14.77%, respectively). In green fruits (Figure 2E), P-36, P-41, P-42, P-43, P-44, P-46, and P-72 obtained the highest antioxidant capacity values (between 47.56 and 59.69%), while lower values (between 15.95 and 20.4%) were for P-35, P-37, P-40, and P-49, which dropped to 8.6% in P-39, which was the lowest value observed in the experiment. In red fruits (Figure 2F), the antioxidant capacity of several landraces ranged from 89.9 to 95.58% (P-39, P-41, P-44, P-46, P-48, P-49, P-50, P-51, P-70). Only five landraces (P-35, P-37, P-42, P-43, P-47) presented low DPPH inhibition (around 66.4 ± 3.5% FW). When comparing green and red fruits, the lowest but statistically significant increase was detected in P-36 (1.5-fold). DPPH inhibition capacity did not significantly change in landraces P-42 and P-43, but they both had the least increased values (1.14- and 1.11-fold, respectively).

As previously mentioned, as all the landraces turned red upon maturity, the increased lycopene inside landraces was evidenced even in those in which this pigment was not recorded in green fruits. However, several landraces are worthy of a special mention, such as P-48 because it had the highest lycopene concentration in red fruits (41.83%), or P-39, P-41, and P-49 with around 27.1%, despite it being absent in the green ones. It is also worth noting P-46 because it exhibited a higher concentration in green fruits but recorded one of the lowest lycopene increments upon maturity (only 2.2-fold).

G1 and G2 fruit were less wrinkled upon maturity (Figure 6D), but pericarp sinuosity increased (Figure 7A). Pedicel persistence with both the stem and, especially, fruit reduced during maturation (Figures 7B and S2B). Maturity appeared to be related to the increased appearance of apical rot (Figure 7C), especially in the G2 fruit, for which the number of affected landraces rose from 1 to 6. This also occurred in P-49. Finally, fruit cracking (Figure 7D) seemed to accentuate with ripening, but only in G2 (rising from 0 to 5 out of 11 in the red fruit).

2.2. Nutraceutical Compounds and Antioxidant Capacity Correlations in Green and Red Fruits

To understand the contribution of different phytochemicals and antioxidant capacity in fruits at both maturity stages, several correlation analyses were carried out with the different combinations of the six traits (Figure 3).



*** Figure 3.** Linear correlation coefficient (r) and its significance for fruit traits (A) in green and (B) red fruits in the collection of the 18 pepper landraces cultivated in Spain. ***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$, and non-significant values for r.

In green fruits, pairwise coefficients showed a positive correlation and statistical significance for five trait combinations (Figure 3A). The highest correlations were related to carotenoid content. The combination carotenoid vs. total Chls obtained the highest r-value (r = 0.655), while the correlation coefficients for carotenoid vs. ascorbic acid and vs. phenols were 0.455 and 0.317, respectively. Despite the low values (around r = 0.27), antioxidant capacity also positively related to phenols (r = 0.27) and ascorbic acid (r = 0.267). Lycopene did not correlate significantly with any trait in green fruits.

In red fruits (Figure 3B), five positive and statistically significant correlations were found for the 15 studied combinations. The two highest pairwise correlations were for phenols vs. chlorophylls and for phenols vs. lycopene (r = 0.420 and r = 0.356, respectively). Lycopene also correlated with carotenoid concentration in mature fruits (r = 0.235). Antioxidant activity and phenol concentration both correlated with ascorbic acid concentration (r = 0.261 and r = 0.272, respectively). In this case, a negative and statistically significant correlation was found between ascorbic acid and lycopene concentration, despite its low r-value (r = -0.012).

2.3. PCA Analysis

The PCA analysis and eigenvalues above one reflected a different pattern in the correlation of the traits in both the green and red fruit (two PCs and three PCs, respectively).

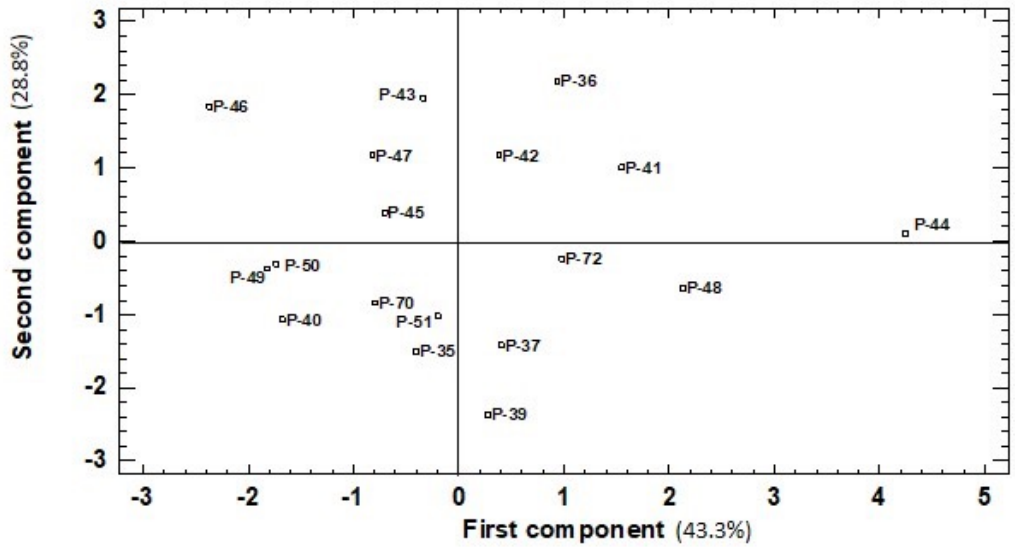
	Component 1	Component 2	Component 3
Green Fruits			
Phenols	0.385	0.458	
Ascorbic acid	0.541	0.050	
Lycopene	−0.247	0.357	
Carotenoid	0.575	−0.027	
Chlorophyll a + b	0.384	−0.499	
Antioxidant capacity	0.142	0.640	
Eigenvalue	2.60	1.73	
Variance explained (%)	43.32	28.82	
Cumulative variance explained (%)	43.32	72.14	
Red Fruits			
Phenols	0.602	0.136	−0.155
Ascorbic acid	0.239	−0.658	−0.220
Lycopene	0.483	0.268	0.379
Carotenoid	0.201	−0.316	0.807
Chlorophyll a + b	0.464	0.350	−0.269
Antioxidant capacity	0.303	−0.504	−0.245
Eigenvalue	2.02	1.22	1.03
Variance explained (%)	33.59	20.31	17.09
Cumulative variance explained (%)	33.59	53.90	70.99

*** Table 2.** Correlation coefficients for each morphological trait for the three first principal components, eigenvalue, and the relative and cumulative proportions of total variance explained by these components, in the collection of the 18 pepper landraces.

In green fruits, the first and second PCs accounted for 43.3% and 28.82% of the total variation for the studied six traits, respectively (Table 2). The first PC correlated positively with all the traits, except for lycopene content. Two traits (ascorbic acid and carotenoid content) showed the highest values for the correlation of the first PC (0.54 and 0.575, respectively). Phenols and chlorophylls presented moderate correlations (around 0.38), while antioxidant capacity had a low value (<0.15). A low value (-0.25) was obtained for the negative correlation of the first PC with lycopene content. When analyzing the second PC, the highest positive correlation was recorded for antioxidant activity (0.64). A moderate positive correlation was found with phenol and lycopene concentrations (0.46 and 0.36, respectively). In this case, two traits presented a negative correlation with the second PC, a moderate value was obtained for chlorophyll content (-0.50) and carotenoid content came very close to zero (-0.027).

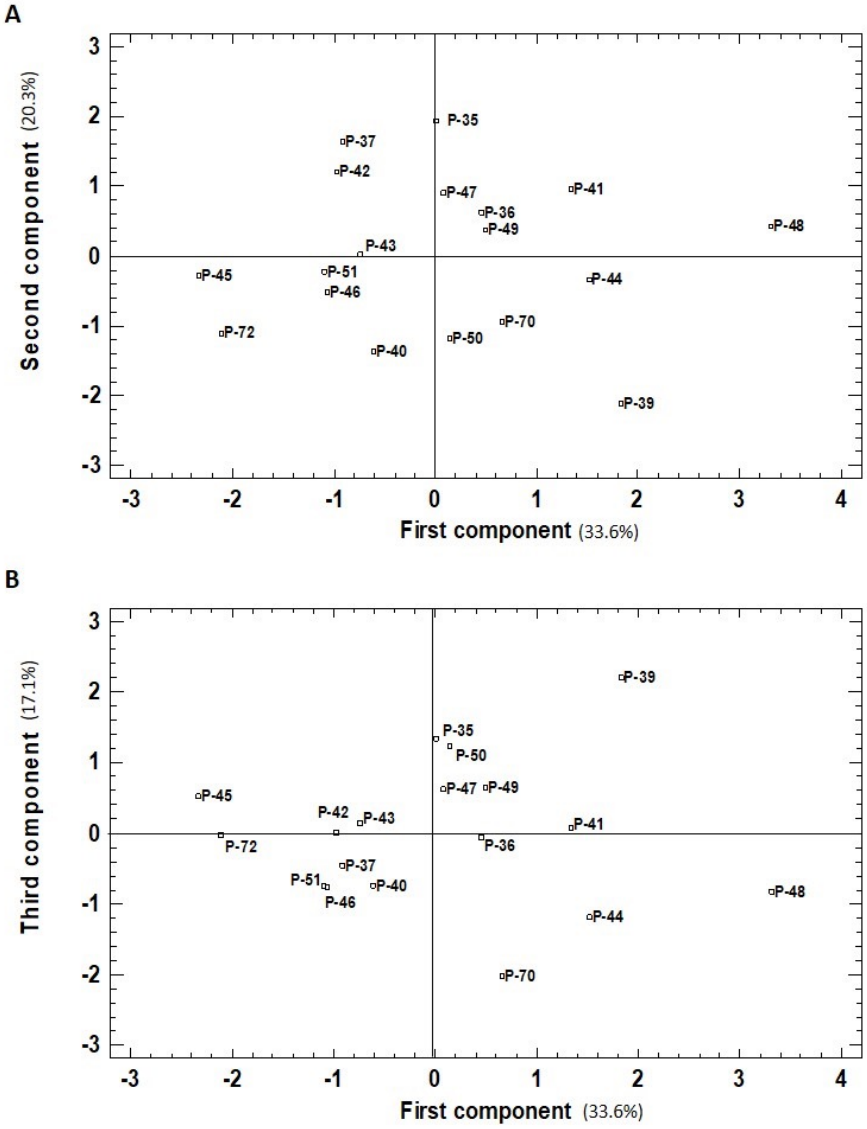
In red fruits, the first, second, and third PCs accounted for 33.6%, 20.3%, and 17.1%, respectively. The first PC correlated positively with all the traits included in the analysis. The highest values (>0.4) for the correlation of the first PC were for phenols, chlorophyll, and lycopene concentration, while moderate correlations (between 0.2 and 0.3) were found with the other traits. The second PC correlated significantly and negatively with ascorbic acid and DPPH (-0.66 and -0.50, respectively), and moderately with carotenoid content (-0.32). This second PC showed a moderate positive correlation (0.35 and 0.27) with the other pigments (chlorophylls and lycopene, respectively) and had a very low value with phenols (0.14). The third component showed a positive correlation with two traits: a strong one with carotenoid content (0.81) and a moderate one with lycopene (0.38). The negative correlation with the other four traits ranged from -0.27 to -0.16.

For both ripening stages, the projection on the PCA plot showed that landraces were widely spread over the area. In green fruits, four to six landraces were found plotted in each quadrant (Figure 4). The highest value for the first PC was recorded for landrace P-44 (Figure 4) and correlated with its top levels for four traits: phenols, ascorbic acid, carotenoids, and chlorophylls, (Figures 1A, 1C, 2A, 2C). P-46, with the lowest value for the first PC, came in the last-but-one place for carotenoids, chlorophylls, and ascorbic acid traits, and the fourth-lowest place was for phenols, albeit not statistically significant compared to the lowest values. Nevertheless, P-46 had one of the highest values for the second PC given its high DPPH and lycopene levels (Figures 1E, 2E). Two more landraces (P-36 and P-43) presented high values for the second PC because of their high phenol concentration and antioxidant capacity (Figures 1A, 1E), but low chlorophyll and lycopene contents (Figures 2C, 2E). The landraces with the lowest value for the second PC (P-35, P-37, and P-39) displayed the least DPPH capacity (Figure 2E) and their phenols concentration (Figure 1A) did not statistically differ from the lowest ones. However, their high Chl concentration (rank 4, 2, and 3, respectively) did not significantly differ from the highest value recorded for P-44 (Figure 2C).



*** Figure 4.** Similarities among green fruits belonging to the 18 pepper accessions evaluated based on six traits (total phenolics, ascorbic acid, carotenoid, lycopene, and chlorophyll content, DPPH scavenging activity) represented the two first components (first component, x-axis; second component, y-axis) of the principal components analysis (43.3% and 28.8% of the total variation, respectively).

The PCA results in red fruits revealed that the first PC separated the landraces with the highest phenols, chlorophyll, and lycopene contents (Figure 5). P-48 obtained the first highest value for this component as it ranked in first place for phenols and lycopene concentrations, and third for chlorophylls. Three other landraces (P-44, P-41, and P-39) with a high value for the first PC also displayed good levels of phenols (rank 2, 3, and 7, respectively), but were not statistically different from the P-48 level (Figure 1B). P-44 had the second-highest value for chlorophylls, and P-41 and P-39 had the second and third highest lycopene contents. The two accessions with the lowest values for the first PC (P-45 and P-72) also had the lowest values for phenols (rank 18 and 16, respectively; Figure 1B), chlorophylls (rank 17 and 18, respectively, Figure 2D), and lycopene (rank 14 and 18, respectively, Figure 1F).



*** Figure 5.** Similarities among red fruits belonging to the 18 pepper accessions evaluated based on six traits (total phenolics, ascorbic acid, carotenoid, lycopene and chlorophyll content, DPPH scavenging activity) represented in (A) the two first components (first component, x-axis; second component, y-axis) of the principal components analysis (33.6% and 20.3% of total variation, respectively); and (B) the first and third components (first component, x-axis; third component, y-axis) of the principal components analysis (33.6% and 17.1% of the total variation, respectively).

As the second PC strongly and negatively correlated with ascorbic acid and DPPH activity, the landraces with the highest values for this component presented very low levels for both these traits (Figure 5A). This was the case of P-35, with the lowest levels of both determinations in red fruits for the whole experiment (Figures 1D, 1F). Three other landraces (P-37, P-42, and P-47) were among the lowest values for phenols (rank 14, 15, and 11, respectively; Figure 1B) and DPPH capacity (rank 15, 14, and 13, respectively; Figure 2F). In contrast, the lowest values for the second PC, recorded for P-39 and P-40, related with their high phenol content in red fruits (rank 1 and 2, respectively, Figure 1B). P-39 also has good levels of carotenoid (rank 1, Figure 2B) and DPPH (rank 8, but with the same significance as the highest values; Figure 2F). The position of P-50 for this second PC was related to its high carotenoid and DPPH capacity, with rank 2 (Figure 2B) and 4 (Figure 2F), respectively.

The third PC of the analysis in red fruits correlated with a high level of carotenoid concentration and moderate lycopene concentration (Figure 5B). The landrace with the highest value for this component (P-39) ranked 1st and 3rd, respectively, for these traits (Figures 2B, 1F). Two other landraces with good levels of the third PC were P-35 and P-50, with rank 4 and rank 2 for carotenoids (Figure 2B), and rank 5 and 6 for lycopene, both respectively (Figure 1F). Instead, P-70 was that with the lowest third PC value and low contents of both traits (rank 15 and 17, respectively).

2.4. Differences between Accession Groups

According to the PCA analysis, the 18 accessions were located on the plot and grouped according to their first/second PCs distribution and positive/negative values (+/-). Groups A (positive for both components, +/+), B (+/-), C (-/-), and D (-/+) were formed. The statistical analysis performed among the four groups detected significant differences in all the studied traits (Table 3).

In the green peppers, the first vs. the second plot (Figure 4) showed that group A stood out for the highest phenol values, which agreed with the fact that it included three of the four accessions with the highest values for this parameter in green fruits (P-36, P-41, P-44; Figure 1A). Group A, together with group B, also showed the highest ascorbic acid values (one of the two landraces with the highest values; P-44; Figure 1C), DPPH (six of seven; P-36, P-41, P-42, P-43, P-44, P-46; Figure 2E). Two landraces in these groups (P-41 and P-44) had the highest carotenoid content compared to the other studied varieties (Figure 2A). In general, group C grouped those landraces whose phenolic, ascorbic acid, and carotenoid contents were very low, and also included four of the seven landraces with the lowest antioxidant capacity. However, landraces P-35 and P-51 stood out for their high chlorophyll concentration in green fruits. Group D included two of the three landraces (P-46 and P-47) with the highest lycopene concentration in green fruits (Figure 1E).

Green Fruits

First/Second PC

	Group															
	A				B				C				D			
Phenols	5.51	±	1.43	a	3.84	±	1.35	b	3.46	±	0.98	b	4.21	±	1.23	b	***
Ascorbic acid	1.66	±	0.31	a	1.70	±	0.45	a	1.20	±	0.33	b	1.34	±	0.29	b	***
Lycopene	0.50	±	0.65	b	0.20	±	0.41	b	0.14	±	0.32	b	1.86	±	2.49	a	***
Carotenoid	7.80	±	2.49	a	7.11	±	1.80	a	5.36	±	1.87	b	5.63	±	1.45	b	***
Chlorophyll a + b	35.92	±	11.61	ab	42.56	±	10.05	a	34.36	±	9.89	bc	28.20	±	8.56	c	**
DPPH	51.89	±	10.18	a	27.07	±	19.11	a	26.55	±	13.03	b	46.12	±	12.97	b	***

Red Fruits

First/Second PC

	Group															
	A				B				C				D			
Phenols	10.04	±	2.13	a	10.00	±	1.60	a	7.77	±	1.08	b	8.86	±	1.96	ab	***
Ascorbic acid	1.96	±	0.58	b	2.68	±	0.62	a	2.27	±	0.64	ab	2.09	±	0.69	b	**
Lycopene	24.96	±	10.56	a	14.60	±	10.12	b	10.01	±	4.76	b	15.82	±	3.23	b	***
Carotenoid	32.01	±	12.24	ab	35.85	±	12.85	a	25.23	±	6.01	b	23.93	±	7.54	b	**
Chlorophyll a + b	1.60	±	1.04	a	1.51	±	1.50	a	0.53	±	0.64	b	1.10	±	0.69	ab	**
DPPH	83.18	±	12.99	b	93.57	±	3.86	a	86.61	±	6.72	b	65.90	±	8.50	c	***

Red Fruits

First/Third PC

	Group															
	A				B				C				D			
Phenols	9.52	±	1.76	b	10.78	±	1.94	a	8.17	±	1.03	c	8.20	±	2.20	c	***
Ascorbic acid	2.07	±	0.70		2.54	±	0.59		2.26	±	0.65		2.11	±	0.67		ns
Lycopene	22.97	±	7.17	a	17.59	±	15.64	ab	10.65	±	5.55	c	14.76	±	2.80	bc	***
Carotenoid	38.03	±	12.48	a	26.95	±	9.36	b	23.58	±	6.50	b	26.67	±	6.40	b	***
Chlorophyll a + b	1.33	±	0.86	a	1.88	±	1.58	ab	0.80	±	0.73	b	0.66	±	0.69	b	**
DPPH	84.49	±	13.45	ab	91.28	±	6.71	a	83.49	±	11.97	b	70.54	±	8.00	c	***

*** Table 3.** Mean values for the fruit traits in the four groups of accessions established by a multivariate PCA in fruits of the collection of 18 pepper landraces. ***, **, *Mindicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$, and non-significant values, respectively. Different letters in each trait indicate significant differences between groups. at $p < 0.05$ using the LSD test

In the red peppers, the first vs. second plot (Figure 5A) showed that groups A and B included five of the eight landraces with the highest phenols content (P-36, P-39, P-41, P-44, P-48; Figure 1B) and 7 of 14 with chlorophyll concentration (all except P-72; Figure 2D). The landraces from group B had the highest ascorbic acid levels (three of six; P-39, P-44, P-70; Figure 1D). The four landraces in this group were among the seven with the highest antioxidant capacity. The three landraces from group D are among the four with the lowest DPPH capacity in the red fruit. From the third vs. the first plot (Figure 5B), group A included five of the seven landraces with the highest carotenoid concentration in red fruits (P-35, P-39, P-47, P-49, P-50; Figure 2B). Groups C and D included those landraces with the lowest concentrations in chlorophyll, six landraces with the lowest phenol values (6 of 10; P-37, P-40, P-45, P-46, P-51; P-72; Figure 1B), and lycopene (five of six; P-40, P-45, P-46, P-51, P-72; Figure 1F).

3. DISCUSSION

The chemical composition, particularly that of nutraceutical compounds, of a vegetable landrace can confer the product added value, which falls in line with increased consumer concern about the nutritional and nutraceutical values of products and their positive relation to human health [16–18]. Thus, the high antioxidant capacity of pepper fruit, together with its marked richness in ascorbic acid, carotene, phenols, xanthophylls, and flavonoids, make it functional food [19–21].

Of all bioactive constituents, ascorbic acid, lycopene, and phenols are of much interest for pepper quality and depend on both landrace and ripening stages. In our study, red fruits presented almost two-fold more phenolic compounds than green ones, as previously reported in other pepper landraces [19], grafted pepper [1,22,23], and other crops such as tomato [24]. The mean values ranged between 6.74 and 11.74 mg g-1 FW in red fruits (Figure 2B) and decreased between 2.88 and 6.25 mg g-1 FW in green ones (Figure 2A), which are higher than those reported by several authors [4,20,25,26] for red peppers, but similar to those found by Chavez-Mendoza et al. [22] and Sun et al. [27]. Shaha et al. [28] reported that a gradual increase in phenolic concentration was observed from green to red ripening. Kevers et al. [29] also reported high levels of total phenols in red, yellow, and green peppers (296, 284, and 215 mg 100 g-1 FW, respectively), which are even higher than those found in spinach, broccoli, cucumbers, and carrots. Conversely, Rodriguez-Burruezo et al. [30] and Vera-Guzmán et al. [31] characterized several landraces from Spain, Mexico, and the USA for bioactive compounds, and obtained a wide range of variation. Navarro et al. [32] did not find any differences in phenols concentration between green and red fruit from cv. Orlando, a "California"-type pepper obtained from a commercial nursery. BlancoRíos et al. [33] discovered that the green bell pepper had the highest total phenol content, but no significant differences between red, yellow, and orange were observed. According to Marín et al. [34], the immature fruit contained the highest phenolic concentrations, while ripe fruit contained the lowest. The intrinsic landrace effect is worth highlighting as we observed that not always do the landraces with the highest total phenols content upon maturity correspond to those with the highest relative increase between green and red fruit. In addition, low phenol levels do not necessarily mean poor antioxidant capacity because this also depends on the phenolic compounds profile, as suggested by some authors [35,36]. In our study, a positive correlation was obtained between both traits in green fruits.

Otherwise, the free radical scavenging abilities of peppers determined by the–DPPH method were 2.29-fold lower for green than red fruits. However, green fruits presented more variability and a mean DPPH inhibition for the diffe-

rent landraces, which ranged from 8.58 to 59.69% (Figure 2E). DPPH inhibition in red fruits ranged only from 63.92 to 95.58% (Figure 2F). These results evidenced that regardless of landrace, red fruits had the highest antioxidant capacity compared to green ones, which coincides with data also reported by other authors in sweet bell pepper cultivars [26,33]. When comparing antioxidant capacity between fruits inside the same landrace, we observed that antioxidant capacity was generally positively influenced by ripening. The most marked increase (10.5-fold) was found in P-39, while no significant increment in antioxidant capacity took place in P-42 and P-43. The influence of maturity processes on DPPH activity has also been reported by Sun et al. [26] who attributed the difference in antioxidant activity between green and red peppers to their different carotenoid, phenolic, and flavonoid contents.

However, the correlation analysis in the whole of fruits only revealed a significant correlation between antioxidant capacity and two parameters (phenolic and vitamin C contents) in green fruits (Figure 3A). Both correlation coefficients were very low ($r = 0.270$ and $r = 0.267$, respectively), which was likely due to the wide range of the features and variability in the landraces herein used. There is no defined trend to correlate antioxidant capacity and phenolic content as different authors' results are extremely variable. Although positive correlations have been determined in date, apple, and pear cultivars [37], nonsignificant interaction has been reported in cereal crops [38]. These differences could be associated with the diverse responses of phenolic compounds in the Folin–Ciocalteau method [39]. Moreover, not all of the many possible phenolic compounds are active radical scavengers or exert an identical matrix effect [40]. The correlation results were more variable in the red fruits (Figure 3B), which suggests that pepper fruit's antioxidant activity may also be attributed to other soluble compounds besides polyphenols. This was the case of, for instance, lipophilic compounds with antioxidant properties (e.g., most carotenoids) that assays usually ignore [35].

We also determined lycopene and total carotenoid content. Although the concentration of lycopene showed a marked variability between plant materials for both ripening stages, it was especially notable in immature fruits as reflected by the coefficient of variation (Table 1). While the low signal recorded in some green extracts suggested a lack of lycopene, three landraces stood out for their relatively high lycopene concentration in green fruits. This was the case of P-45, P-46, and P-47 (Figure 3E) with 1.80, 5.50, and 3.84 mg g-1 FW, respectively. All the landraces had high lycopene levels in red fruits (around 27.38- fold higher than green ones; Table 1), even those with a low or practically no signal in immature fruit (Figure 3E). Although this was an expected result, several varieties are worth mentioning. One is P-48, which had the highest lycopene concentration in red fruits (41.83 mg g-1 FW). Landraces P-35, P-41, P-39, and P-49 (26.05 ± 1.73 mg g-1 FW) had the second-highest levels of this compound, although it was practically absent in green fruits. In contrast, P-46 showed a minor lycopene increment upon maturity despite its highest level in green fruits. All these results support the notion of the wide variability in the behavior of landraces toward the maturity process, as previously mentioned, and agrees with other studies conducted with many commercial pepper cultivars, such as bell and California-type pepper plants, and with other species such as tomato [1,22,32,41]. In addition, the possible origins of lycopene must also be considered as the bibliography does not clarify a single one, but suggests that ripening-dependent lycopene accumulation may derive from either β-carotene synthesis inhibition or an alternative ripening-specific pathway like the 1-deoxy-D-xylulose-5-phosphate pathway [42,43]. In the tomato, it is not known whether the progressive transition in pulp color from red to orange–yellow, which signifies over-ripening, derives from the conversion of accumulated lycopene into β-carotene, or from senescence-related lycopene degradation [43,44]. Nevertheless, our results about lycopene are most interesting and

relevant for several reasons: (1) the most efficient quencher of singlet oxygen and free radicals among carotenoids [45]; (2) unlike β-carotene, lycopene is used entirely as an antioxidant because it is not transformed into vitamin A [46]; (3) it is well-known that lycopene is not sensitive to heat treatment, such as ascorbic acid and, thus, remains unaltered even after cooking fruit [41].

Regarding the total carotenoids concentration, although we observed differences in landraces, this compound apparently depends less on plant material, which is the exact opposite of lycopene. In general terms, most landraces presented a similar carotene concentration in both green (around 6.35 µg g-1 FW) and red (about 30.58 µg g-1 FW) fruit. Only P-44 in green fruits and P-39, P-47, and P-50 in red fruits are highlighted for their high carotene content in relation to the corresponding average value for each maturity stage (between 2- and 3.5-fold higher, respectively). Differences between plant materials have been reported in other studies carried out with chili and sweet pepper landraces [31,47] and grafted pepper plants [22,23,48]. We observed a marked dependence for carotenoids on fruit ripeness, which was stronger in fully ripe than in immature fruits. Carotene concentration was around 4.2-fold higher in red than in green fruits in most landraces, but this behavior rose between 7.36- and 11.51-fold in three of the 18 landraces (P-39, P-49, P-50). This finding in mature fruits has been reported by several authors in mature fruit, specifically commercialized paprika, and sweet and hot chili peppers [32,33,49,50] and is related to not only the increment in the number of total carotenoids, but also to the change in the pigment profile [4,34]. During pepper ripening, chloroplast pigments (chlorophylls and carotenoids like lutein and neoxanthin) disappear, while carotenoid chromoplast pigments (β-carotene and xanthophylls like capsanthin) are synthesized [34,51–53]. Our mean values of total chlorophylls in all the landraces support these works because the green fruits obtained a high total chlorophyll content in them all (between 27.10 µg g-1 FW and 51.65 µg g-1 FW), which lowered in the red fruits by more than 95% as a consequence of the ripening process, and concomitantly with an increment in carotenoids. Conversely, Sun et al. [40] have reported a similar carotene concentration between green and red bell peppers. However, this result is not comparable to ours because the fruit belongs to different varieties.

Interestingly, some reports have observed substantial variations for many carotenoids in colored fruit from traditional landraces, which suggests reservoirs of useful traits, including those that might be able to contribute to improved human nutrition and new breeding opportunities [54]. However, Tripodi et al. [55] demonstrated a wide range of bioactive compounds in pepper, including carotenoids, were highly dependent on the environmental component.

In short, we observed that the increase in both total carotenoid and lycopene content did not necessarily display the same tendency. This was supported by the lack of a significant correlation between carotenoids and lycopene in green fruits, and by the statistically significant, but low, r coefficient in red ones (r = 0.235, Figure 3B). Similar results have been reported by Chávez-Mendoza et al. [22], who found an increase in both fruit antioxidant capacity and β-carotene content, but not in lycopene content, with two pepper cultivars when they were grafted onto rootstock "Terrano".

Finally, the variability of vitamin C between landraces suggests genotype dependence for this trait in peppers. The average ascorbic acid concentration in the different landraces ranged from 1.07 to 2.23 mg g-1 FW in green fruits (Figure 2C), and from 1.45 to 3.16 mg g -1 FW in red ones (Figure 2D). These values are similar to others reported in studies conducted in commercial sweet pepper and chili cultivars [56,57] and other traditional pepper ecotypes [30].

Higher values have also been recorded by Osuna-García et al. [58] and Palma et al. [59] in their studies carried out in sweet pepper and chili varieties, and also by Antonius et al. [26], Orobiyi et al. [60], and Ribes-Moya et al. [4] in traditional landraces, especially red fruit.
When comparing green and red fruits, some landraces are highlighted because their ascorbic acid concentration depended on the ripening stages, with statistically higher values in the mature ones than in the immature ones. This is the case of landraces P-39, P-43, P-46, P-47, P-50, and P-70, whose mean vitamin C content was between 1.63- and 2.18-fold higher in red than in green fruits, and P-40 with the highest increment (2.95-fold) throughout the experiment. The landraces with the highest values in immature fruits did not obtain the highest relative increments upon ripening. For instance, P-40 and P-70 had the lowest values throughout the experiment in green fruits (1.07 and 1.33 mg g-1 FW, respectively), but the highest ones (3.15 and 2.89 mg g-1 FW, respectively) upon maturity. We also found that some landraces, such as P-35 and P-49, had low ascorbic acid levels for both maturity stages and displayed a non-significant increment for mature fruits. All these findings indicate a marked dependence of this compound on landrace, which agrees with other studies carried out on traditional pepper varieties [30,61,62]. The relation between ascorbic acid and fruit ripening, particularly the increment of this nutraceutical compound in fresh peppers as fruit advanced, has been previously described in commercial cultivars [32–34,36,58,63,64]. Palma et al. [59] did not find any differences in ascorbate concentration upon maturity in Melchor and Piquillo varieties, but they did in Padrón and Alegría. These authors pointed out a likely stabilizing role of ascorbate to assure capsaicinoids levels when oxidized by peroxidases during maturity.

Moreover, the vitamin C levels found in these landraces in both red and green fruits support the notion that pepper is one of the crops with the highest levels of this compound. According to the FAO (Food and Agricultural Organization) and the WHO (World of Health Organization) recommendations, fruits with more than 1.13 mg g-1 FW are rich in vitamin C (as in all red peppers fruits and most green ones; Figure 2C,D) and can be considered potential vitamin C sources, as previously reported by Orobiyi et al. [60]. In fact, pepper had similar levels to those of other vegetables that are well-known for their vitamin C content, such as kale or broccoli [65], or even more than double that found in fruits like citrus, grapevine, kiwi fruit, or strawberry [66]. As an average intake of 25 mg of ascorbic acid is enough to meet the daily intake of this vitamin in humans [67], 50 g of fresh fruit intake from most local analyzed landraces would provide such requirements, even when unripe. Thus, the nutraceutical value of pepper might not be questioned. With the correlation analysis (Figure 4), we found a varied range of correlations among the studied nutraceutical compounds. Generally, ascorbic acid and carotene contents are likely the two nutraceutical compounds with the most marked relations with the other compounds. In green fruits, carotenoids positively correlated with phenols, chlorophylls, and ascorbic acid. This last compound was also positively related to antioxidant capacity in immature fruits. The acceptable concentration of carotenoids found even in green fruits suggests an active synthesis-degradation route of β-carotene toward other successor compounds, such as capsanthin or capsorubin, which are exclusive of pepper and have antioxidant properties, or vitamin A, an essential nutrient not produced by the human body, but one essential for growth and development, epithelial tissue maintenance, reproduction, and proper visual system functioning in the regeneration of photoreceptors [68].

DPPH positively correlated with phenols in green fruits, but not in red fruits, while antioxidant capacity and phenols seem to be related more to ascorbic acid concentration in mature fruits, which indicates a strong dependence on

the fruit maturity processes. Interestingly, the highest correlations in mature fruits were found between phenols and pigments, particularly chlorophylls and lycopene. The results reported by other authors are variable. Studies have shown a positive direct correlation between antioxidant potential and phenolic compounds content in pepper and other crops, such as grapes, eggplants, olives, and citrus, among others [19,26,47,69]. Araujo et al. [70] reported that antioxidant activity correlated positively with several other variables, ranging from a strong to a weak correlation for chlorophyll a, chlorophyll b, titratable acidity, carotene, total phenols, flavonoids, anthocyanins, and ascorbic acid. Materska and Perucka [38] highlighted the influence of phenolic profile and its relation to the number and positions of hydroxyl groups in aromatic rings, esterification or the free form of the analyzed compounds, and methoxy substituents in the ortho position to OH. However, Chavez-Mendoza et al. [1] reported an inverse correlation between antioxidant capacity and lycopene.

Finally, as the four groups formed by the PCA analysis were composed of landraces of different origins and morphological characteristics, this suggested that a wide diversity exists for the traits studied in the groups. Thus, in our collection, we identified four landraces of interest in immature fruits: P-44, which is interesting for its high content in carotenoids, chlorophylls, phenols, and ascorbic acid; P-46, with good antioxidant capacity and high lycopene content; P-36 and P-43 with high phenols concentration and antioxidant capacity. However, P-48 is of much interest for its high phenols and lycopene content in red fruits. P-44 and P-41 also presented good levels of phenols, chlorophylls, and/or lycopene upon maturity. Other landraces of interest in ripe fruits were: P-39, as highlighted by its phenols, carotenoid, and DPPH values; P-35 and P-50 for their high carotenoid and lycopene contents, and excellent DPPH capacity. The PCA results generally showed the usefulness of the multivariate analysis for classification in studies with a great number of landraces and is a powerful tool in breeding programs for pepper and other crops used to describe and/or select cultivars with high added value [47,70–73].

4. MATERIALS AND METHODS

4.1. Plant Material

The plant material for this study consisted of 18 pepper landraces ***(C. annuum)*** that represent the pepper germplasm collection of Valencia (Spain). Landraces were provided by the COMAV and IVIA. Table 4 provides the numerical code, passport identification, fruit shape description, and origin of each landrace. Figure 6 complements this table. Seeds were sown on 7 March 2019 in 104-hole seed trays filled with enriched substrate for germination.

Code	Germplasm Code	Fruit Description	Origin
P-35	(1) BGV005087	Rectangular shape, blocky and with four shoulders, and locule marked	Fanzara, Castellón, Spain
P-36	(1) BGV005035	Irregular, rectangular-conical shape but inconsistent pattern, slightly marked shoulders	Chelva, Valencia, Spain
P-37	(1) BGV005097	Triangular shape and truncated apex	Castillo de Villamalefa, Castellón, Spain
P-39	(1) BGV005115	Triangular shape and truncated apex	Alicante, Spain
P-40	(1) BGV005125	Rounded-elongated triangular shape and truncated apex	Elda, Alicante, Spain
P-41	(1) BGV014141	Elongated (horn type), very slightly marked shoulders	Vinaròs, Castellón, Spain
P-42	(1) BGV014145	Elongated (horn type), very slightly marked shoulders	Almenara, Castellón, Spain
P-43	(1) BGV014146	Elongated (horn type), very slightly marked shoulders	Castellón de la Plana, Castellón, Spain
P-44	(1) BGV016188	Elongated (horn type), very slightly marked shoulders	Guardamar del Segura, Alicante, Spain
P-45	(1) BGV005064	Triangular shape and truncated apex	Ademuz, Valencia, Spain
P-46	(1) BGV005085	Rectangular shape, blocky and with four shoulders, and locule marked	Onda, Castellón, Spain
P-47	(1) BGV005040	Rounded-elongated triangular shape and truncated apex	Siete Aguas, Valencia, Spain
P-48	(1) BGV005034	Elongated (horn type), very slightly marked shoulders	Chelva, Valencia, Spain
P-49	(1) BGV005046	Ball-like shape with very slightly marked shoulders	Benissa, Alicante, Spain
P-50	(1) BGV005116	Triangular shape and truncated apex	Rojales, Alicante, Spain
P-51	(1) BGV014553	Rectangular shape, blocky and with four shoulders, and locule marked	Tales, Castellón, Spain
P-70	(2) IVIA 70	Rounded-elongated triangular shape and apex truncated	Moncada, Valencia, Spain
P-72	(2) IVIA 72	Elongated (horn type), very slightly marked shoulders	Canal de Navarrés, Valencia, Spain

*** Table 4.** Abbreviation, germplasm collection code, fruit shape description, and origin of the 18 pepper varieties included in the study. Plant material was provided by: (1) the Valencian Institute for the Conservation and Improvement of Agrobiodiversity (COMAV, Spain) and (2) the Valencian Institute for Agricultural Research (IVIA, Spain).

*** Figure 6.** Pepper fruits in different maturity stages (red and green) obtained from the cultivated landraces. The size of the grid cells in the fruit pictures is 1 cm × 1 cm.

4.2. Greenhouse Experiment

The experiment was conducted from May to September 2019 in an unheated plastic multi-span greenhouse in the experimental field belonging to the IVIA (Valencia, Spain; latitude: 39.58951793357715, longitude: -0.3955507278442383). The soil composition within 20 cm depth was 68% sand, 11% clay, and 21% silt (sandy-clay loam) containing 0.61% organic matter, 0.051% total N, less than 8 mg kg−1 of P, 301 mg kg−1 of K, and 2.87 meq·100 g−1 of assimilable Mg. Soil electrical conductivity was 0.290 dS m−1 and pH was 8.1.

Plants were transplanted on 9 May a2019 and grown under greenhouse conditions in single rows (110 cm apart) with a 50 cm spacing between each plant. Each landrace consisted of six plants. Irrigation satisfied 100% of the crop evapotranspiration (ETc), as described in Penella et al. [74], performed with a drip system. Nutrients were applied by the irrigation system at a rate (kg ha-1) of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO, and 35 MgO, as recommended by Maroto [75]. The average range of minimum and maximum temperatures during the experiment was 12–24 °C for May, 15–28 °C for June, 19–32 °C for July, 19–32 °C for August, and 18–29 °C for September.

4.3. Nutraceutical Compounds and Antioxidant Capacity

4.3.1 Sample Preparation

From each landrace, eight randomized fruits were harvested from the end of July to mid-September. Fruit samples were taken from four independent plants, four replicates for each landrace, in each maturity stage: green and red. Fruits were washed and prepared; a 3 cm-wide longitudinal section was transversally cut at the fruit midpoint and homogenized (Kinematica Polytron PT 3100, Lucerne, Switzerland) at 15,000 g for approximately 2 min. Final extracts were divided into aliquots of 0.3 and 1 g, frozen in liquid N_2, and stored at −80 °C until further determinations.

4.3.2 Total Phenolic Analysis

Phenolic content was analyzed according to Dewanto et al. [76] with modifications. Briefly, a 1 g aliquot of sample extract was homogenized in 4.0 mL of 80% (v/v) methanol, vortexed, incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min, and then revortexed. Samples were centrifuged at 10,000 g for 15 min at 4 °C and the supernatant was used for the analysis. Total phenolic content was determined by the Folin-Ciocalteau colorimetric method. A 10 µL aliquot of the supernatant was mixed with 115 µL of distilled water, 125 µL of Folin-Ciocalteau reagent, and 1.25 mL of $NaHCO_3$ (7%) and then incubated in a dark cupboard for 90 min. Solution absorption was measured at 760 nm in a spectrophotometer (Uvikon XS, Bio-Tek, Winooski, VT, USA). The blank solution without extract was used for calibration. Each measurement was compared to a standard curve of gallic acid (GA) and total phenols were expressed as mg of GA equivalent g-1 FW.

4.3.3 Ascorbic Acid Concentration

Ascorbic acid content was spectrophotometrically determined as described by Kampfenkel et al. [77]. Briefly, 0.3 g of each sample was homogenized and adjusted to a 2 mL volume with 6% (w/v) trichloroacetic acid (TCA). Samples were centrifuged at 10,000 g for 3 min and the supernatant was used for the analysis. Then, 0.05 mL of the homogenate was mixed with 0.05 mL of 10 mM DTT and 0.1 mL of 0.2 M phosphate buffer (pH 7.4). Samples were incubated for 15 min at 42 °C. Afterwards, 0.05 mL of 0.5% (w/v) NEM (N-ethylamide) was added and incubated for 1 min at room temperature. Next, 0.25 mL of 10% (w/v) TCA, 0.2 mL of H_3PO_4 4% (w/v), 0.2 mL of 2-2′-dipyridyl, and 0.1 mL of 3% (w/v) $FeCl_3$ were added to the previous solution. They were all incubated together in a water bath for 40 min at 42 °C. Solution absorption was measured at 525 nm in a spectrophotometer (Uvikon XS, Bio-Tek). Ascorbic acid was expressed as mg g-1 FW.

4.3.4 Antioxidant Capacity Measurements

Antioxidant capacity was measured following the method reported by Brand-Williams et al. [78] with modifications. Sample extract (1 g) was homogenized in 4.0 mL of 80% methanol (v/v), incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min, and then vortexed. Samples were centrifuged at

10,000 g for 15 min at 4 °C and 10 µL of phenolic extract was added to 990 µL of a solution containing 3.12 × 10−5 M of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 80% methanol. The absorbance at 515 nm was measured against a blank solution (80% methanol without extract) after a 30-min reaction time at room temperature in the dark (optimized for the highest antioxidant concentrations in the extract) using a spectrophotometer (Uvikon XS, Bio-Tek). The results were expressed as the percentage reduction of the initial DPPH.

4.3.5 Carotenoids and Chlorophyll Concentration

Carotenoids (Car) and total chlorophyll (Chl) (a and b) concentration were determined spectrophotometrically as described by Porra et al. [79]. Next, 1.5 mL of 80% acetone (v/v) was added to sample extracts (0.3 g) and centrifuged at 7000 g for 10 min. The supernatant was used for the analysis. Solution absorption was measured at 663, 648, and 470 nm in a spectrophotometer (Uvikon XS, Bio-Tek). Then, 80% acetone (v/v) was used as the blank solution (without extract). The chlorophyll (a and b) and carotenoids content of the extracts were calculated using the following equations:

$$\text{Chl a} = 12.25 \times \text{Abs663} - 2.55 \times \text{Abs648}\ (\mu g\ mL{-}1) \quad (1)$$

$$\text{Chl b} = 20.31 \times \text{Abs648} - 4.91 \times \text{Abs663}\ (\mu g\ mL{-}1) \quad (2)$$

$$\text{Car} = [(1000 \times \text{Abs470} - 1.82\ \text{Chl a}) - (85.02 \times \text{Chl b})]/198\ (\mu g\ mL{-}1) \quad (3)$$

Chlorophylls and carotenoids were expressed as µg g−1 FW.

4.3.6 Lycopene Concentration

Lycopene was extracted from pepper fruit using a hexane:ethanol:acetone (2:1:1; v:v:v) mixture following the method of Adejo et al. [80] with modifications. Sample extract (10 mg) was dissolved in 1 mL of distilled water and vortexed in a water bath at 30 °C for 1 h. Then, 8.0 mL of the hexane, ethanol, and acetone mix was added, capped, and re-vortexed, followed by incubation in a dark cupboard for 60 min. Subsequently, 1 mL of distilled water was added to each sample, vortexed once more, and left until it separates into phases. Care was taken to ensure that the formed bubbles had fully disappeared. The cuvette was rinsed with the upper layer of one of the blank samples before using more fresh blank samples (distilled H_2O without extract) to zero the spectrophotometer at 503 nm. Three milliliters of the upper layers of the lycopene samples was taken and their absorbance at 503 nm wavelength was read by a spectrophotometer (Uvikon XS, Bio-Tek). The lycopene content of extracts was expressed as mg g−1 FW.

4.4. Statistical Analysis

The results for the nutraceutical compounds and antioxidant capacity parameters were subjected to a one-way analysis of variance (ANOVA) using Statgraphics Centurion XVII (Statistical Graphics Corporation 2014) with landrace taken as the factor of the analyses. Each ripening state (green and red fruits) was separately analyzed. The

results were expressed as mean ± standard deviation. Means were accepted as being significantly different at a 95% confidence interval ($p \leq 0.05$). The mean, maximum and minimum values, coefficient of variation, and F-ratio of the nutraceutical traits in green and red fruits were calculated. An analysis of the correlation between the different traits in each ripening state was calculated as the linear correlations between the individual samples of each accession ($n = 72$) and the correlation coefficient (r) was obtained.

A principal component analysis (PCA) using Statgraphics Centurion XVII (Statistical Graphics Corporation 2014) was carried out for the standardized values using pairwise Euclidean distances among landraces means to assess the relations between genotypes. The correlation coefficients for each fruit trait for the first three principal components (PCs), the extracted eigenvalues, and relative and cumulative proportions of total variance explained by these components, were calculated. Two-dimensional (2D) scatter plots (first vs. second in the green fruits, and first vs. second and first vs. third in red fruits) were prepared based on a distance matrix for the PCs to visualize the relationship explaining the traits. From the PCs scatter plots, four groups of accessions were established for each ripening state with a different profile for the studied traits. Groups A (positive for both components, +/+), B (+/−), C (−/−), and D (−/+) were formed. The signification of differences among groups of landraces was evaluated by a one-way ANOVA.

5. CONCLUSIONS

From the analysis of the nutraceutical compounds of 18 pepper landraces, we conclude that:

(1) Landrace type and harvest pepper period can be chosen to achieve the desired optimal fruit quality. Mature fruits are related to high vitamin and carotenoids contents, for which landrace P-39 is remarkable, while green ones are associated with high polyphenol contents, traits that highlight the importance of accession P-44;

(2) Nutritional characterization of pepper landraces can contribute to promote their use and increase their added value. This work could be of practical use as a start point in breeding programs for growing antioxidant-rich varieties, especially with good levels of vitamin C and total phenolics, and for enhancing the conservation of traditional varieties that are in danger of genetic erosion.

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CHAPTER 05

PHENOTYPING LOCAL EGGPLANT VARIETIES: COMMITMENT TO BIODIVERSITY AND NUTRITIONAL QUALITY PRESERVATION

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Abstract
Given the little variability among commercialised eggplants mainly in developed countries, exploring and structuring of traditional varieties germplasm collections have become a key element for extending ecotypes and promoting biodiversity preservation and consumption. Thirty-one eggplant landraces from Spain were characterised with 22 quantitative and 14 qualitative conventional morphological descriptors. Landraces were grouped based on their fruit skin colour (black-purple, striped, white and reddish). Landraces B7, B20 and B24 were left out for their distinctive fruit characteristics. Wide variation for plant, leaf, flower and fruit phenology traits was observed across the local landraces, and fruit descriptors were considered the most important ones. In a second experiment, landraces, B14, B16 and B17 were selected to determine fruit quality. By contemplating the benefits provided by antioxidants and sugars for human health, pulp antioxidant capacity, total phenolic, ascorbic acid, carotenoid, flavonoid and total sugar content were determined. Significant differences were observed across these three landraces, and B14 was highlighted for its antioxidant properties, while B17 stood out for its high sugar content. B16 did not stand out for any traits. The results indicate the wide variability in eggplants for their phenotypic and nutritional characteristics, which emphasises the importance of traditional varieties as the main source of agricultural biodiversity.

Keywords
eggplant, landrace, biodiversity, phenotype, antioxidants, nutraceutical value.

1. INTRODUCTION

Nutritional habits have vastly changed, and the consumption of fruit and vegetables has grown thanks to the abundance of health-promoting compounds found in them [1]. They are provided a wide range of minerals [2], proteins [3,4], fiber [5] and antioxidants [6,7]. However, fruit and vegetables appreciation has mainly increased due to the beneficial effects associated with dietary antioxidants [8].
Eggplant is a common annual vegetable crop grown in subtropical and tropical areas [9]. It is one of the most important vegetable crops, and 1.85 million cultivated hectares (ha) worldwide are used to grow it (with a production of 55 million tonnes). It has a huge economic impact in Africa, Europe, and especially Asia, which harvests more than 90% of the total eggplant production. It is particularly important in China and India. Spain is the world's tenth largest producer of this vegetable [10].

Although most commercial varieties are purple [11,12], eggplants are known for being highly variable in fruit colour, shape and size. A representative part of this diversity is found among traditional varieties. Landraces are crop varieties that have been differentiated by farmers through a historical selection process and they represent great genetic heritage as a source of agricultural biodiversity [13]. These local varieties are better adapted to specific agroclimatic conditions, and they are suitable for new agriculture kinds, such as organic production [14,15].

The fruit of the eggplant not only contains proteins, minerals, dietary fiber, minerals of interest as potassium, calcium, magnesium, sodium, iron [16], but is also enriched in polyphenols, including phenolic acids such as chlorogenic acid, caffeic acid and p-coumaric acid [17,18], and flavonoids, including trace quantities of flavonols and a high content of various acylated and nonacylated anthocyanins specially in purple-coloured varieties [19]. Also is appreciated for its content in other antioxidants as ascorbic acid [20] and vitamins, especially vitamin P [21], although has low provitamin A carotenoid content as compared to other solanaceous crops such as tomatoes and peppers [6]. These bioactive compounds are responsible for higher functional properties of eggplant [19], as they neutralise reactive oxygen species (ROS) by reducing lipid peroxidation and damage to cellular organelles [9,20], and provide antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory properties in humans [22–25].

In relation to nutritional concerns, the eggplant ***(Solanum melongena L.)*** has become a highly appreciated crop. Of 120 vegetables evaluated to determinate their antioxidant capacity, eggplant was ranked in the top ten for its oxygen radical absorbance capacity, mediated mainly by fruit´s phenolic constituents [12,26]. Nevertheless, a wide natural variation in antioxidant capacity has been found between eggplant landraces [12,26,27]. It is known that the quantity and quality of phenols present in fruit is conditioned by the environment, soil type, and also growing and storage conditions [28–30]. Therefore, having a detailed description of the characteristics and nutraceutical quality of traditional eggplant varieties should attach considerable interest giving the high phenotype biodiversity that can be found in these landraces.

The main challenge of crop genetic selection lies in the reliability of available phenotypic data [31].The rapid development of genomics, has offered crop breeders the ability to develop high yielding and stress tolerant plants, but the ability

to acquire high yielding phenotypic data hinders this opportunity [32].

Currently, non-destructive phenotyping technologies, like hyperspectral imaging or multispectral fluorescence, are of great interest as they allow predicting the content of many nutraceuticals compounds without damaging the plant itself [33,34]. Most of these facilities collect information in controlled environments using robotics and automatic image acquisition and analysis [31]. However, although this type of non-destructive technique can estimate a wide range of internal biochemical data in a short time, information on the ability of biochemical reflectance indices to quantify many compounds is still lacking [35]. Compared to hyperspectral studies, more progress has been made in fluorescence methods. The multi-channel fluorescence systems with multi-colour excitation have been significantly improved and the commercial devices became available and widely applied [36]. However, these technologies are still under active development [37].

This work seeks to revalue traditional eggplant varieties from the Valencian Community (Spain) as the biodiversity of the territory has been severely diminished by widely cultivated commercial hybrids. In this context, 31 eggplant landraces were selected from the plant resources stored in the genebank of the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV, Valencia) and the Valencian Institute for Agrarian Research (IVIA, Valencia). Even if the majority of the selected eggplants had black-purple or striped skin, other less common varieties were included in the assay, since having a high degree of diversity was advantageous, both for their possible use in breeding programs and for promoting their conservation. Once the phenotypic data had been collected, the nutritional and nutraceutical characteristics of three selected landraces were determined in order to gain benefits derived from their use.

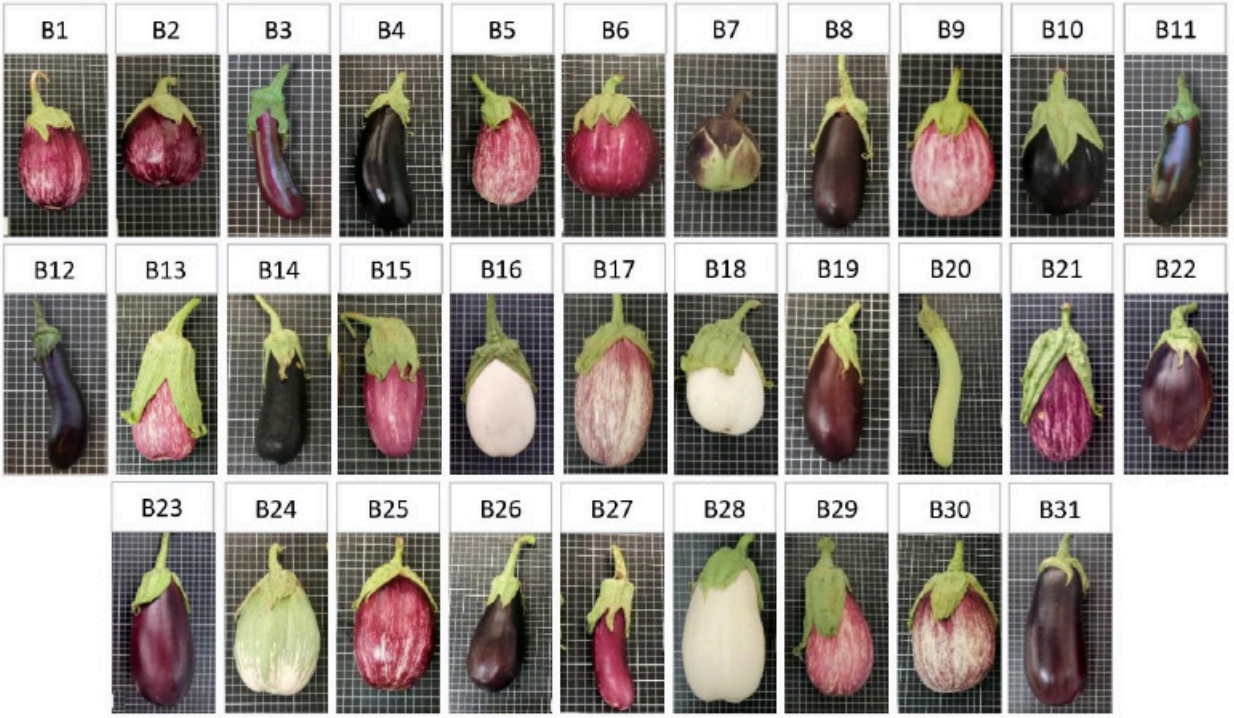
2. MATERIALS AND METHODS

2.1. Plant material and soil experiment

The work herein presented is divided into two main experiments: one focuses on phenotypic characterisation and the other on nutritional quality. They were carried out in two consecutive years (2019 and 2020).

Abreviation code	Genbank code	Group	Original Location
B1	BGV005769	G2	Alcira, Valenica, Spain[1]
B2	BGV005770	G2	Gandía, Valencia, Spain[1]
B3	BGV005771	G4	Gandía, Valencia, Spain[1]
B4	BGV005774	G1	Jaraco, Valencia, Spain[1]
B5	BGV005776	G2	Valencia, Spain[1]
B6	BGV005778	G2	Orihuela, Alicante, Spain[1]
B7	BGV005781	-	Benijofar, Alicante, Spain[1]
B8	BGV005780	´G1	San Fulgencio, Alicante, Spain[1]
B9	BGV005783	G2	Aspe, Alicante, Spain[1]
B10	BGV005784	G1	Novelda, Alicante, Spain[1]
B11	BGV005785	G1	Elche, Alicante, Spain[1]
B12	BGV005787	G1	Mutxamel, Alicante, Spain[1]
B13	BGV005788	G2	Benejama, Alicante, Spain[1]
B14	BGV005789	G1	Gandía, Valencia, Spain[1]
B15	BGV005790	G2	Orihuela, Alicante, Spain[1]
B16	BGV015751	G3	Alacuás, Valencia, Spain[1]
B17	BGV008284	G2	Moncada, Valencia, Spain[1]
B18	BGV015630	G3	Torreblanca, Castellón, Spain[1]
B19	BGV015745	G1	Benimasot, Alicante, Spain[1]
B20	BGV015762	-	Alcudia de Crespins, Valencia, Spain[1]
B21	BGV015847	G2	Onteniente, Valencia, Spain[1]
B22	BGV015763	G1	Onteniente, Valencia, Spain[1]
B23	BGV015848	G1	Onteniente, Valencia, Spain[1]
B24	BGV015849	-	Jaraco, Valencia, Spain[1]
B25	BGV015850	G2	Jaraco, Valencia, Spain[1]
B26	BGV015834	G1	Jaraco, Valencia, Spain[1]
B27	BGV015835	G4	Jaraco, Valencia, Spain[1]
B28	BGV015836	G3	Jaraco, Valencia, Spain[1]
B29	BGV014500	G2	Villarreal, Castellón, Spain[1]
B30	B-81	G2	Gandía, Valencia, Spain[2]
B31	B-76	G1	Alginet, Valencia, Spain [2]

*** Table 1.** Abbreviation, germplasm collection code, group (based on eggplant skin colour, G1= black-purple, G2 = striped, G3 = white, G4 = reddish purple) and origin of the 31 eggplant landraces used in the study. Plant material was provided by the: (1) Valencian Institute for the Conservation and Improvement of Agrobiodiversity (COMAV, Spain); (2) Valencian Institute for Agricultural Research (IVIA, Spain).



*** Figure 1.** Pictures of the 31 cultivated eggplant landraces (***S. melongena*** L.) provided by the Germplasm Banks from the COMAV and the IVIA (Spain). The size of the grid cells in the fruit pictures is 1 cm × 1 cm.

2.1.1 Experiment 1: Phenotyping study

For the first-year experiment, 31 eggplant landraces were sown on 5 March 2019 and seedlings (8 plants per landrace) were planted on 2 May 2019. Plants were grown in single rows placed 120 cm apart leaving 60 cm between each plant. Irrigation of plants satisfied 100% of the crop evapotranspiration (ETc) as described in Penella et al. [38] with a drip system. Nutrients were applied via the irrigation system at a rate (kg ha-1) of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO and 35 MgO, as recommended by Maroto [39]. The average range of the minimum and maximum temperatures for the first-year experiment was 12-24 ºC for May, 15-28 ºC for June, 19-32 ºC for July and 19-32 ºC for August [40]. Measurements were taken when fruits reached the commercial maturity, along July and August depending on the landrace (plants around 2.5-3 months after transplant). Data for plant, leaf and flower traits were taken from eight

independent plants, which gave 8 replicates per landrace. Fruit traits were measured in ten different fruits which were representative of the landrace, which equals 10 replicates per variety.

2.1.2 Experiment 2: Fruit quality study

During the second-year experiment, landraces B14, B16 and B17 were chosen as being representative of the main fruit colour groups, namely, G1: black-purple, G2: striped and G3: white, to analyse fruit quality and to, thus, provide verified information on their added value and to facilitate their trade. Landraces from G4 group and outliers (B7, B20 and B24) were not considered due to their reduced number of representatives and unsuitable qualities for cultural practices and market observed in experiment 1 (high number of calix prickles, undesirable growth habit, low number of flowers per inflorescence, intense leaf pilosity...). Seeds were sown on 7 March 2020 and seedlings (10 plants per landrace) were planted on 13 May 2020. Agronomic culture practices were similar as in the first-year experiment. The average range of the minimum and maximum temperatures was 11-31 ºC for May, 14-31 ºC for June, 18-33 ºC for July and 19-34 ºC for August [40]. Fruits were harvested when reached the commercial maturity, along July and August depending on the landrace (plants around 2.5-3 months after transplant). For each case, two fruit samples were taken from ten independent plants, which gave 20 replicates per landrace.

2.2. Agromorphological characterisation and data collection

The quantitative and qualitative agromorphologic data from thirty-six phenotypic traits (Table 2) measured in plants, stem, leaves, flowers and fruit were scored and classified according to the International Board for Plant Genetic Resources descriptors (IBPGR 1990) for eggplant.

Descriptors	Unit/Scale
QUANTITATIVE	
Plant Morphology	
Length	cm
Width	cm
Branch density	1.Very scarce (≤ 2) 3.Scarce (approx. 5) 5.Intermediate (approx. 10) 7.Dense (approx. 20) 9.Very dense (> 30)
Leaf Morphology	
Length	cm
Width	cm
Pedicel length	mm
Pedicel thickness	mm
Colour	*L**
	*a**
	*b**
Dented leaf blade	1.Very weak 3.Weak 5.Medium 7.Strong 9.Very strong

Blistering	1.Very weak 3.Weak 5.Medium 7.Strong 9.Very strong
Pilosity (per cm^{-2})	1.(< 20) 3.(20 - 50) 5.(50 - 100) 7.(100 - 200) 9.(> 200)
Flowers	
Number per inflorescence	
Fruit	
Number of colours in commercial maturity	
Length	cm
Width	cm
Length / width	
Weight	g
Calyx length	cm
Calyx lenght total lenght ratio	%
Number of calyx prickles	

Table 2. List of the descriptors used for phenotyping according to the International Board for Plant Genetic Resources descriptors (IBPGR 1990) for eggplant.

Descriptors	Unit/Scale
QUANTITATIVE	
Plant Morphology	
Length	cm
Width	cm
Branch density	1.Very scarce (≤ 2) 3.Scarce (approx. 5) 5.Intermediate (approx. 10) 7.Dense (approx. 20) 9.Very dense (> 30)
Leaf Morphology	
Length	cm
Width	cm
Pedicel length	mm
Pedicel thickness	mm
Colour	*L**
	*a**
	*b**
Dented leaf blade	1.Very weak 3.Weak 5.Medium 7.Strong 9.Very strong
Blistering	1.Very weak 3.Weak 5.Medium 7.Strong 9.Very strong
Pilosity (per cm^{-2})	1.(< 20) 3.(20 - 50) 5.(50 - 100) 7.(100 - 200) 9.(> 200)
Flowers	
Number per inflorescence	
Fruit	
Number of colours in commercial maturity	
Length	cm
Width	cm
Length / width	
Weight	g
Calyx length	cm
Calyx lenght total lenght ratio	%
Number of calyx prickles	

*** Table 2.** List of the descriptors used for phenotyping according to the International Board for Plant Genetic Resources descriptors (IBPGR 1990) for eggplant

2.2.1 Leaf and fruit colour

The colour of eggplant leaves was determined by placing the colorimeter (Minolta Colorimeter model CR-400) on the central part of the adaxial face. Two independent colour measures were taken in each plant, which gave 16 data per landrace. L* (lightness), a* (red/green chromatic coordinates) and b* (yellow/blue chromatic coordinates) measures were recorded in order to determine leaf colour. Eggplant peel colour was assigned by researchers visually, being thus considered a qualitative trait.

2.3. Fruit quality determinations

The percentage of dry weight (DW), pulp colour, antioxidant capacity, and total phenolics, flavonoids, ascorbic acids, carotenoids and sugar contents, were analysed in the mid-part of the pulp of harvested fruits in B14, B16 and B17 to determine if there were significant differences among them.

2.3.1 Fruit dry material

In order to establish the percentage DW in fruits, the fresh weight (FW) of eggplants was recorded. Samples were dried at 65 ºC for 72 h in a laboratory oven. The DW percentage was calculated as (DW/FW) × 100.

2.3.2 Pulp colour

Fruit slides (10 mm wide [41]) were cut transversally in the central part of the eggplant and colour in the inner pulp was measured by colorimeter (Minolta Colorimeter model CR-400). One measure in the central part of the sample was taken in each fruit, which gave 20 data per landrace. L* (lightness), a* (red/green chromatic coordinates) and b* (yellow/blue chromatic coordinates) measures were recorded in order to determine pulp colour immediately after fruit eggplants were cut

2.3.3 Nutraceutical compounds and antioxidant capacity

Sample extract
Nutraceutical compounds and antioxidant capacity were analysed in the pulp of eggplant fruit. Samples were peeled, cut into pieces and homogenised (Polytron PT 3100, Kinematica AG.,) at 15,000 g for approximately 1 min. Final extracts were divided into aliquots of 2 g, frozen in liquid nitrogen and stored at -80 ºC until further determinations were made.

Antioxidant capacity measurements
Antioxidant capacity was measured following the method reported by Brand-Williams et al. [42] with a few modifications. The sample extract (1 g) was homogenised in 4.0 mL 80% (v/v) methanol, incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab) at medium intensity for 30 min and then vortexed. Samples were centrifuged at 10,000 g at 4 ºC for 15 min. Then 10 µL of the extract were mixed with 990 µL of a solution composed of 3.12 × 10-5 M of 2,2-diphenyl-^{1}-picrylhydrazyl (DPPH) in 80% methanol. The decrease in absorbance at 515 nm contrasted with a

blank solution containing 80% methanol with no extract after a 30-minute reaction time at room temperature and in the dark using a spectrophotometer (Uvikon XS, Bio-Tek). Antioxidant capacity was expressed as the percentage reduction of the initial DPPH absorption in extracts.

Total phenolic content

Phenolic content was analysed as described by Dewanto et al. [43] with some adjustments. The sample extract (1 g) was mixed with 4.0 mL of 80% (v/v) methanol, vortexed and incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab) at medium intensity for 30 min. Samples were centrifuged at 10,000 g at 4 ºC for 15 min. The total phenolic concentration was determined following the procedure of (Singleton and Rossi [44] based on the Folin-Ciocalteu colorimetric method. Then 10 µL of the supernatant were mixed with 115 µL of distilled water, 125 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, Co.) and 1.25 mL of $NaHCO_3$ (7%). Afterwards the mix was incubated at room temperature for 90 min in complete darkness. The absorption of the solution was measured at 760 nm in a spectrophotometer (Uvikon XS, Bio-Tek). A blank solution with no extract was used for calibration. Total phenolic concentration was compared to a standard curve using gallic acid (120-600 mg L^{-1}). Total phenolic content was expressed as mg gallic acid equivalent (GA) g-1 FW.

Total flavonoid content

Flavonoid content was measured following the method reported by Du et al. [45] with some modifications. Briefly, 1 g of sample extract was homogenised in 4.0 mL of 80% (v/v) methanol, incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab) at medium intensity for 30 min and then vortexed. Samples were centrifuged at 10,000 g at 4 ºC for 15 min. Then 0.3 mL of the supernatant were mixed with 3.4 ml of 30% ethanol, 0.15 ml of $NaNO_2$ 0.5 M and 0.15 mL of $AlCl_3$. Next $6H_2O$ 0.3 M was added and vortexed. Samples were incubated for 5 minutes at room temperature. Afterwards 1 mL of NaOH 0.1 M was added to the mixture. The absorption of solution was measured at 506 nm in a spectrophotometer (Uvikon XS, Bio-Tek). Total flavonoid concentration was compared to a standard curve using rutin (Merck Co.) as the standard (4.7-300 mg L^{-1}). Flavonoid content was expressed as mg rutin equivalent g^{-1} FW.

Ascorbic acid concentration

Ascorbic acid content was spectrophotometrically determined according to Kampfenkel et al. [46]. The sample extract (0.3 g) was mixed with 2 mL of 6% (w/v) TCA (trichloroacetic acid). Samples were centrifuged at 10,000 g for 3 min. Next 0.05 mL of the supernatant were mixed with 0.05 mL of 10 mM DTT and 0.1 mL of 0.2 M phosphate buffer (pH 7.4). Samples were incubated for 15 min at 42 ºC. Subsequently, 0.05 mL of 0.5% (w/v) NEM (N-ethylamide) were added to the mix and incubated for 1 min at room temperature. Later 0.25 mL of 10% (w/v) TCA, 0.2 mL of H_3PO_4, 0.2 mL of 4% (w/v) 2,2´-dipyridyl and 0.1 mL of 3% (w/v) $FeCl_3$ were added to the previous solution. The mixture was incubated in a water bath for 40 min at 42 ºC. The absorption of solution was measured at 525 nm in a spectrophotometer (Uvikon XS, Bio-Tek). Ascorbic acid was expressed as mg AsA 100 g^{-1} FW.

Carotenoid concentration

The carotenoid concentration was determined spectrophotometrically as reported by Porra et al. [47]. The sample extract (0.3 g) was mixed with 1.5 mL of 80% acetone (v/v) and centrifuged at 7,000 g for 10 min. The supernatant was used for the analysis. The absorption of solution was measured at 663 nm, 648 nm and 470 nm in a spectro-

photometer (Uvikon XS, Bio-Tek) and 80% acetone (v/v) was used for the blank solution. The carotenoid concentration of samples was calculated using Equation (1), and then expressed as μg g^{-1} FW:

(1) Carotenoids (μg mL^{-1}) = [(1000 × Abs470 − 1.82 × Chl a) − (85.02 × Chl b)]/198

Where Chl a and Chl b were the chlorophyll a and b contents, respectively, and were calculated by Equations (2) and (3); Abs is the absorption of samples at a specific wavelength (nm):

(2) Chl a (μg mL^{-1}) = 12.25 × Abs663 − 2.55 × Abs648

(3) Chl b (μg mL^{-1}) = 20.31 × Abs648 − 4.91 × Abs663

2.3.4 Total soluble sugar content

Soluble sugar content was spectrophotometrically determined according to Calatayud et al. [48] with several modifications. The sample extract (0.3 g) was mixed with 15 mL of 80% ethanol (v/v), which was previously heated. The mixture was incubated in a water bath for 10 min at 85 ºC and then vortexed. Samples were centrifuged at 10,000 g at 23 ºC for 10 min. The resulting supernatant was reserved in a flask. This same process was repeated 2 more times by adding hot ethanol to the mixing tube. The ethanol present in the reserved supernatant was then evaporated by a rotary evaporator (R-210, Buchi) at 50 ºC. The sugar concentrate was diluted in 100 mL of distilled water and filtered to be reserved in a volumetric flask for 24 h at 4 ºC. Next 0.5mL of this solution was mixed with 2 mL of distilled water and placed on ice. Once cooled, 5 mL of 4 μM anthrone (Acros Organics B.V.B.A.) solution, diluted in 96% (v/v) sulphuric acid, were added to each tube. Samples were incubated in a water bath for 7.5 min at 85 ºC and then placed on ice for 30 min. The absorption of solution was measured at 630 nm in a spectrophotometer (Uvikon XS, Bio-Tek). Total sugar concentration was compared to a standard curve using a diluted (1:25) stock solution of 55.6 μM glucose and 70 μM sodium benzoate as the standard. Total sugar content was expressed as g glucose equivalent 100 g^{-1} FW.

2.4. Statistical analysis

The results obtained from the evaluated parameters underwent a one-way ANOVA analysis in Statgraphics Centurion XVII (Statistical Graphics Corporation 2014) using the selected landraces as the factor of analyses. The results were expressed as the mean ± standard deviation (SD). The means were accepted as being significantly different at a 95% confidence interval ($p \leq 0.05$).

Principal component analysis (PCA) was run for the standardised values using pairwise Euclidean distances among accession means to determinate any relations between genotypes. The extracted eigenvalues, and the relative and cumulative proportions of the total variance explained by the first three components, were calculated. Two-dimensional (2D) scatter plots (the first vs. the second and the first vs. the third principal components) were prepared based on a distance matrix for the principal components to visualise the relation that explained traits. For the PCA analysis, the phenotypic data pertaining to the 31 landraces was considered together.

Furthermore, two correlation analyses, in which the individual samples of each accession were subjected to linear regression and the correlation coefficients (r), were completed among the: 1) selected phenotypic quantitative traits of each landrace (n = 31); 2) dry weight, pulp colour and antioxidant traits of the selected landraces (B14, B16, B17).

3. RESULTS

3.1. PCA analysis of phenotyping traits

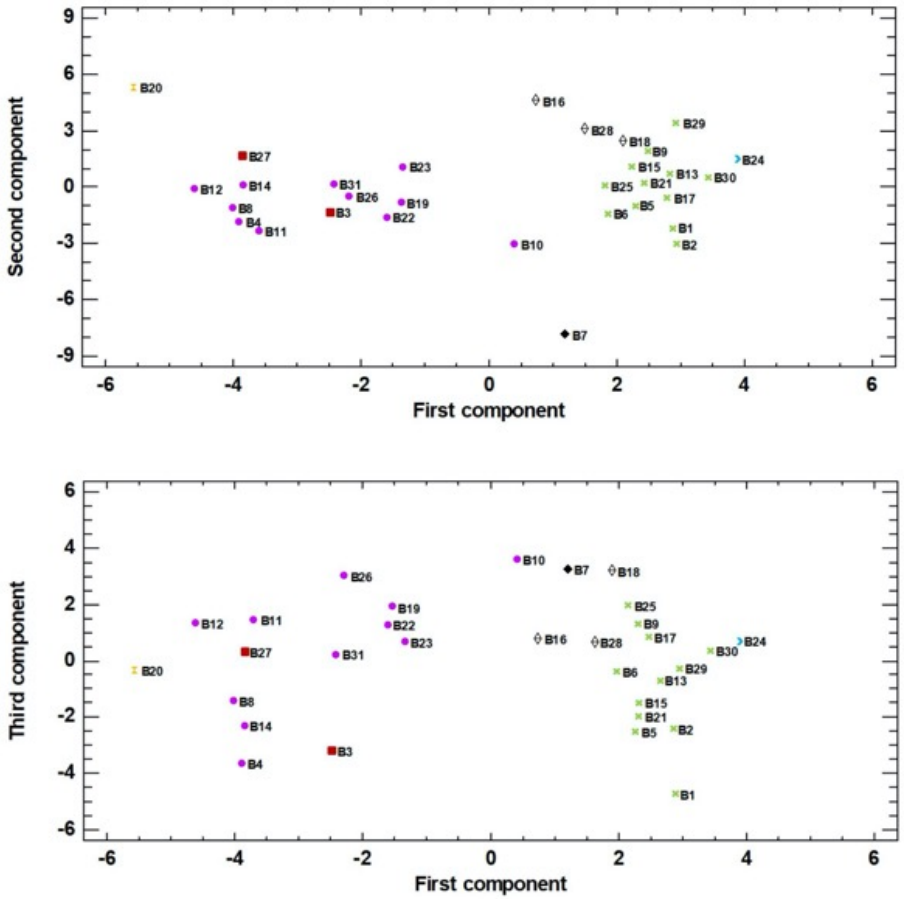
The PCA analysis and those eigenvalues above 1 reflected a different pattern in the correlation of eggplants (Table 3). Nine principal components were determined, which described around 80% of the variability between landraces. Here it is only shown the distribution of landraces based on the most significant principal components; the first, second and third components of the PCA, which respectively accounted for 23.33%, 16.73% and 12.04% of the total variation for the studied traits.

	First Principal Component	Second Principal Component	Third Principal Component
Pl Growth habit	0.216	0.194	
Pl Branch density		-0.159	-0.199
Pl Height	-0.225		
Pl Width			-0.164
S Relative internode length		-0.173	-0.230
S Anthocyanin pigments intensity		-0.334	
S Pilosity		-0.227	
L Puffiness	0.211		
L Dental leaf blade		0.291	
L Thorn presence			-0.339
L Pilosity			
L Length			-0.240
L Width	0.213		-0.294
L Pedicel length		-0.180	-0.274
L Pedicel thickness			-0.351
L Colour parameter *L**		0.304	
L Colour parameter *a**		-0.309	
L Colour parameter *b**		0.342	
Fl Corolla colour	-0.204		
Fl Number per inflorenscence			
Fr Longitudinal shape	-0.280		-0.176
Fr Curvature	-0.214		-0.209
Fr Cross section shape			0.208
Fr Apex shape	0.260		
Fr Colour distrib. at maturity	0.253		
Fr Pulp colour	-0.257		
Fr Colour predominant maturity		-0.272	
Fr Secondary colour (if any)	0.174		
Fr Number of colours	0.193		
Fr Length	-0.188	0.204	-0.222
Fr Width	0.280		
Fr Length/width ratio	-0.252		-0.215
Fr Weight	0.212		
Fr Calyx length			
Fr Calyx lenght/total lenght	0.172		0.186
Fr Calyx prickles number	0.182		
Eigenvalue	8.400	6.022	4.336
Variance explained (%)	23.334	16.728	12.043
Cumulative variance explained (%)	23.334	40.062	52.105

*** Table 3.** Correlation coefficients for each morphological trait for the three first principal components, eigenvalue, and relative and cumulative proportion of the total variance explained by these components in the collection of the 31 eggplant landraces. Only the correlations with absolute 0.150≤ are listed. Pl: Plant, S: Stem, L: Leaf, Fl: Flower, Fr: Fruit.

The first component principally correlated with fruit traits. All the correlations were moderate, and the strongest positive relations were observed with fruit width, apex shape and skin colour distribution upon maturity. Negative correlations were found with fruit longitudinal shape, pulp colour and the length-width ratio. Therefore the darkest and longest eggplants with obscure pulp were placed to the left of the plot, while the widest and lightest eggplants with a striped/mottled skin colour distribution and a whitish pulp were placed to the right of the plot. In line with this, when analysing the second component, the highest correlations were recorded for the leaf and stem traits. In particular, positive correlations were established with colour parameters L* and b* and dental leaf blade, while negative correlations were found for stem anthocyanin pigments intensity and leaf colour parameter a*, among others. So the landraces whose leaves had an intense light-green colour and with very lobed margin were placed in the upper part of the plot, while the landraces with dark-green leaves with soft margins and absence of anthocyanins on the stem were placed in a lower position. The third component of the PCA analysis showed that the correlations with fruit descriptors followed the same pattern as that observed for the first component. In contrast, negative correlations were established with some leaf descriptors: width, presence of prickles, pedicel thickness and pedicel length. In each group described according to the fruit criteria, those landraces with wider leaves, lacking prickles and long pedicels were placed in the upper position in the plot.

The projection on the PCA plot (Figure 2) showed how accessions spread widely over the area. In general, the landraces that were similar in fruit skin colour and shape were placed together, which highlights the importance of both traits. According to this information, several groups were arranged based mainly on fruit skin colour: G1 = black-purple, G2 = striped, G3 = white, G4 = reddish purple. The dark and striped skin eggplants were clearly separated in the plot. The white landraces remained close to the striped ones because of their globular shape, while the reddish ones remained near the black-purple ones given their dark skin colour and elongated shape. Notwithstanding, it was considered necessary to differentiate groups G3 and G4 for their distinctive fruit traits. Landraces B7, B20 and B24 were not included in any of these groups in PCA analysis because of their distinctive fruit morphology.



*** Figure 2.** The principal component analysis (PCA) for the 31 eggplant accessions based on the traits used for phenotyping represented in A) the two first components (first component, X-axis; second component, Y-axis) of the PCA (23.34% and 16.73% of total variation, respectively) and B) the first and third components (first component, X-axis; third component, Y-axis) of the PCA (23.34% and 12.04% of total variation, respectively). Groups, arranged based mainly on fruit skin colour (G1 black-purple, G2 = striped, G3 = white, G4 = reddish purple), are represented in the plot: G1 (●), G2 (✖), G3 (◊) and G4 (■). Outliers B7 (♦), B20 (x) and B24 (›) are also expressed in the figure.

In the plot corresponding to first and second components (Figure 2A), the landraces from G2, G3 and B24 were located furthest to the right according to the correlations determined in the first component. In contrast, landrace B20 was located on the left of the graph because its fruits ranked first for the both fruit-length and width-length ratios. B20 was located in the top position of the plot because its leaves obtained higher values for colour parameters L* and b*, and the lowest value for colour parameter a*. In contrast, landrace B7 remained at the bottom of the plot given the strong anthocyanin pigmentation on the stem in addition to having the lowest values for leaf parameters L* and b*. G1 and G4 were also located on the left of the plot, principally for their elongated shape and their uniform-mottled skin colour distribution. B10 was slightly separated for having the lowest fruit length/width ratio.

The plot projecting the landrace distribution based on the first and third principal components (Figure 2B) did not differ that much from the previous one. In this case however, the varieties of each group appeared somewhat more dispersed given the leaf morphology effect. Landraces B10, B7, B18 and B26 appeared at the top of the graph. B10 stood out for its globous leaves, and B7 and B18 lacked prickles on leaves and displayed a very short pedicel length. B26 stood out for presenting very thin leaves with fine pedicels. Conversely, B1 was located at the bottom of the plot for its wide leaves with thick pedicels and for also presenting the most marked presence of prickles.

3.2. Phenotypic differences between eggplant landraces

Significant differences were found among the average values of all the eggplant groups for the majority of the considered quantitative traits (Table 4). The individual data for each landrace of these groups is shown in the Supplementary Data (Tables S1-2). All the qualitative data is found in Figures 3-5.

Table 4. Variation parameters for the conventional morphologic quantitative descriptors in the 31 local eggplant landraces cultivated in Spain. Statistics were performed by the formed groups based on fruit skin colour; G1 = black-purple, G2 = striped, G3 = white, G4 = reddish purple. Data belonging to outliers (B7, B20, B24) is also shown. Values represent the mean, range, coefficient of variation (CV, %), F-ratio and significance (***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$), for the conventional morphological descriptors studied in cultivated eggplants (n = 8 for plant, leaf and flower traits and n = 10 for fruit traits).

Landrace	G1					G2			
Descriptors	**Mean**	**Range**	**CV (%)**	**F-ratio**		**Mean**	**Range**	**CV (%)**	**F-ratio**
Plant									
Length	94.92	58 - 128	16.85	15.3***		78.18	58 - 109	13.48	9.10***
Width	108.94	80 - 155	13.64	2.97**		113.75	89 - 143	11.02	3.88***
Branch density	5.54	4.90 - 7	9.93	11.06***		5.60	5 - 8	10.81	3.84***
Leaf									
Length	20.22	11 - 27.90	14.10	2.58*		21.58	14.5 - 30	14.33	1.84
Width	12.71	8.50 - 21	21.26	5.17***		16.81	10 - 24.50	18.50	4.63***
Pedicel length	8.72	3.50 - 16	31.70	4.97***		8.82	4 - 15	23.14	4.41***
Pedicel thickness	5.53	3.35 - 9.10	19.04	5.32***		7.00	4 - 10.75	21.53	26.75***
Dented leaf blade	3.44	1 - 7	48.90	28.95***		6.23	4 - 8	15.89	18.33***
Blistering	2.90	1 - 5	24.85	16.05***		3.58	1 - 7	55.61	170.99***
Pilosity (per cm^{-2})	5.80	3 - 7	23.91	79.8***		5.42	3 - 7	26.80	200 <***
Leaf *L**	29.96	26.22 - 34.58	4.99	5.94***		29.68	23.96 - 33.71	6.09	7.76***
Leaf *a**	-6.55	-9.87 - -4.83	|12|	9.01***		-7.09	-8.68 - -4.77	|11.78|	14.2***
Leaf *b**	9.54	7.26 - 12.93	11.88	9.5***		9.96	6.80 - 13.28	11.83	5.9***
Flowers									
Number per inflorescence	1.27	1 - 2	35.15	24.74***		1.36	1 - 3	38.92	56.26***
Fruit									
Number of colours in commercial maturity	1.31	1 - 2	35.48	134.43***		2.03	1 - 3	10.21	1.9*
Length	14.95	7.30 - 26	27.16	19.92***		13.42	8.50 - 19.50	18.63	16.04***
Width	6.50	3.98 - 11.15	25.44	34.97***		8.17	5.48 - 11.69	18.46	9.55***
Length / width ratio	2.52	1.02 - 4.82	31.64	11.98***		1.67	1.08 - 2.92	22.31	13.03***
Weight	216.07	111.27 - 581.65	47.40	18.14***		328.34	112.04 - 808.30	43.63	8.87***
Calyx length	7.60	4.40 - 15.20	24.90	8.58***		8.84	4.30 - 20.70	40.88	10.59***
Calyx lenght total lenght ratio	53.58	24 - 102.68	38.30	35.33***		64.70	28.21 - 152.21	32.76	4.80***
Number of calyx prickles	8.25	0 - 38	107.17	3.37***		20.94	0 - 73	80.06	7.86***

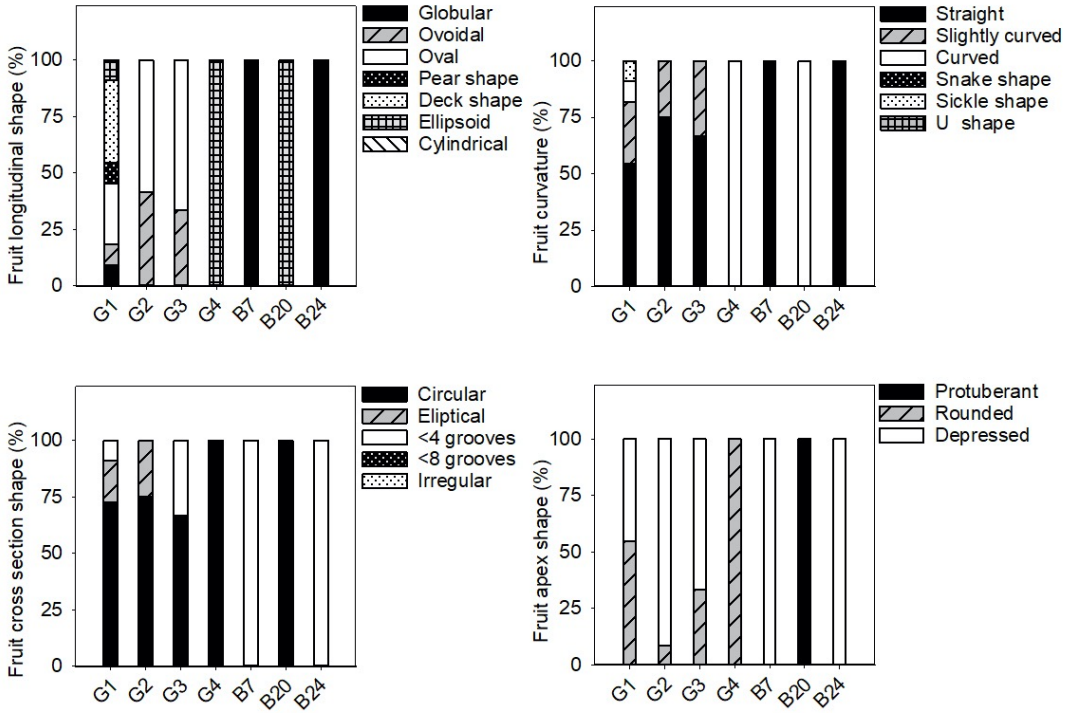
Landrace	G3				G4			
Descriptors	**Mean**	**Range**	**CV (%)**	**F-ratio**	**Mean**	**Range**	**CV (%)**	**F-ratio**
Plant								
Length	72.71	51 - 98	21.20	28.04***	93.78	86 - 109	6.71	2.14
Width	99.38	57 - 126	15.89	11.53***	99.81	84 - 118	9.85	3.61
Branch density	5.04	3 - 7	15.22	6.45**	6.00	5 - 7	12.17	14.00**
Leaf								
Length	20.37	18 - 24.50	10.39	4.58	20.25	16 - 24.60	12.78	8.90**
Width	15.14	10.90 - 22	18.06	5.10	14.31	10 - 20.50	22.78	18.26***
Pedicel length	6.95	5.50 - 9	15.74	2.93	7.38	5 - 10	20.04	13.37**
Pedicel thickness	6.52	5 - 7.90	12.48	3.64*	6.00	4.40 - 8.60	23.27	32.62***
Dented leaf blade	4.67	4 - 6	10.35	3.71*	4.63	2 - 7	41.68	43.81***
Blistering	5.32	4 - 8	26.20	12.52***	3.31	3 - 5	18.18	5.65*
Pilosity (per cm^{-2})	5.35	3 - 7	33.17	82.29***	5.63	3 - 7	28.20	56.47***
Leaf *L**	30.40	26.05 - 34.58	6.29	5.29**	29.68	27.27 - 33.25	4.78	5.72*
Leaf *a**	-7.57	-10.10 – -5.86	|11.48|	9.46***	-6.74	-9.17 - -5.23	|12.62|	0.16
Leaf *b**	10.84	8.61 - 14.29	12.77	5.04*	9.34	7.74 - 12.36	12.22	2.31
Flowers								
Number per inflorescence	1.65	1 - 2.10	29.45	7.82*	1.23	1 - 2	35.63	2.54
Fruit								
Number of colours in commercial maturity	1.00	-	0.00	1.00	1.00	-	0.00	1.00
Length	13.79	10 - 21	16.23	3.57*	15.83	13.4 - 22	14.18	1.71
Width	8.54	5.86 - 12.18	20.74	34.78***	4.10	3 - 5.2	17.89	0.35
Length / width ratio	1.68	1.12 - 2.36	22.17	29.24***	4.03	2.91 - 4.92	13.77	0.06
Weight	349.17	121.90 - 791.40	37.53	9.57***	126.15	66.3 - 208.53	30.67	0.69
Calyx length	8.62	5.50 - 12.50	23.06	10.89***	8.23	4.7 - 12.5	22.87	0.29
Calyx lenght total lenght ratio	63.37	35.26 - 100	26.15	5.82***	49.17	40.46 - 55.97	9.97	20.01**
Number of calyx prickles	22.73	0 - 57	81.49	7.44**	5.89	0 - 16	86.37	0.20

Landrace	B7		B20		B24	
Descriptors	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Plant						
Length	83.13	79 - 91	86.00	66 - 106	88.83	81 - 105
Width	99.13	93 - 103	105.17	98 - 108	135.57	121 - 150
Branch density	5.63	5-6	5.00	-	6.00	-
Leaf						
Length	22.50	19.50 - 26	19.70	18 - 21.90	20.00	17 - 25
Width	15.44	12 - 17.50	13.86	12.50 - 15	14.79	13.50 - 17
Pedicel length	7.75	6 - 11	5.64	5 - 6	9.00	4.90 - 14
Pedicel thickness	5.80	5.15 - 6.70	5.76	4.90 - 6.08	5.41	4.70 - 6.30
Dented leaf blade	5.00	-	5.14	4 - 6	6.00	-
Blistering	1.00	-	5.43	4 - 6	3.29	3 - 4
Pilosity (per cm^{-2})	7.00	-	5.00	-	7.00	-
Leaf *L**	25.16	19.67 - 30	32.49	28.26 - 36.95	31.30	28.84 - 33.45
Leaf *a**	-3.72	-8.19 - 0.61	-7.99	-9.22 - -6.26	-7.61	9.55 - -6.46
Leaf *b**	7.18	3.36 - 11.44	12.39	8.23 - 15.10	10.73	9.89 - 11.13
Flowers						
Number per inflorescence	2.63	2 - 3	1.00	-	1.00	-
Fruit						
Number of colours in commercial maturity	1.00	-	2.00	-	3.00	-
Length	6.14	5.60 - 6.80	22.11	14.70 - 26.90	11.71	8.10 - 14.30
Width	7.11	6.35 - 8.16	3.72	3.05 - 4.44	9.04	7.36 - 9.81
Length / width ratio	0.87	0.75 - 0.95	6.17	5.77 - 7.15	1.36	1.09 - 1.64
Weight	112.89	83.37 - 146.80	120.22	70.27 - 186.02	341.42	231.83 - 465.69
Calyx length	6.94	6.20 - 7.70	5.89	5.40 - 6.50	8.00	5.80 - 11.30
Calyx lenght total lenght ratio	110.86	95.59 - 120.69	25.98	23.66 - 28.93	68.89	50.74 - 93
Number of calyx prickles	2.63	0 - 5	3.50	0 - 8	4.50	0 - 11

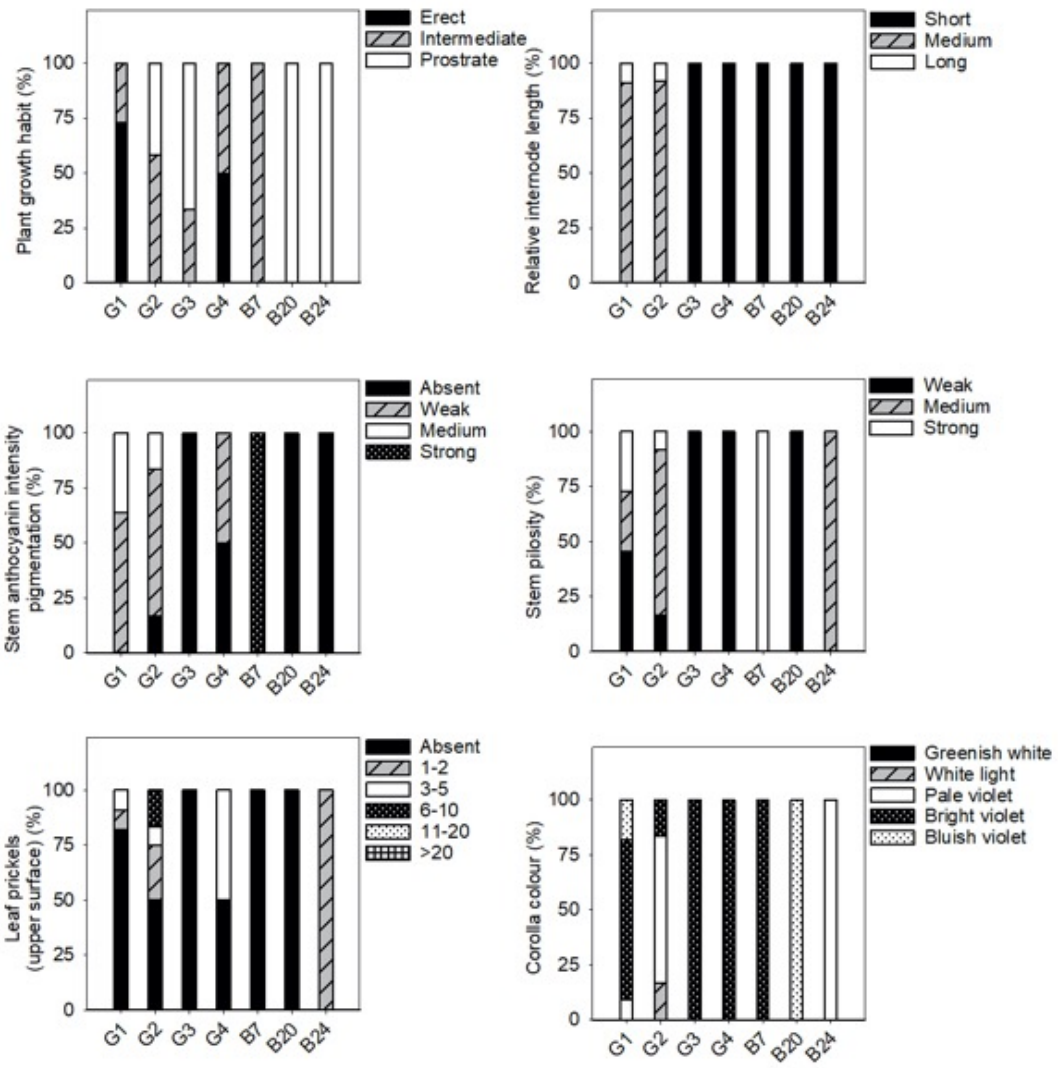
*** Table 4.** Variation parameters for the conventional morphologic quantitative descriptors in the 31 local eggplant landraces cultivated in Spain. Statistics were performed by the formed groups based on fruit skin colour; G1 = black-purple, G2 = striped, G3 = white, G4 = reddish purple. Data belonging to outliers (B7, B20, B24) is also shown. Values represent the mean, range, coefficient of variation (CV, %), F-ratio and significance (***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$), for the conventional morphological descriptors studied in cultivated eggplants (n = 8 for plant, leaf and flower traits and n = 10 for fruit traits).

In general, the fruit purple-black eggplant varieties (G1) were characterised by an erect plant growth habit, medium relative internode length, weak stem anthocyanin pigmentation, strong leaf pilosity and bright violet flowers. The fruits themselves stand out for their oval, pear or deck shape, and were much longer than they were wide, with a rounded or depressed apex, no curvature, white to greenish pulp and their long, but not very prickly calyx.

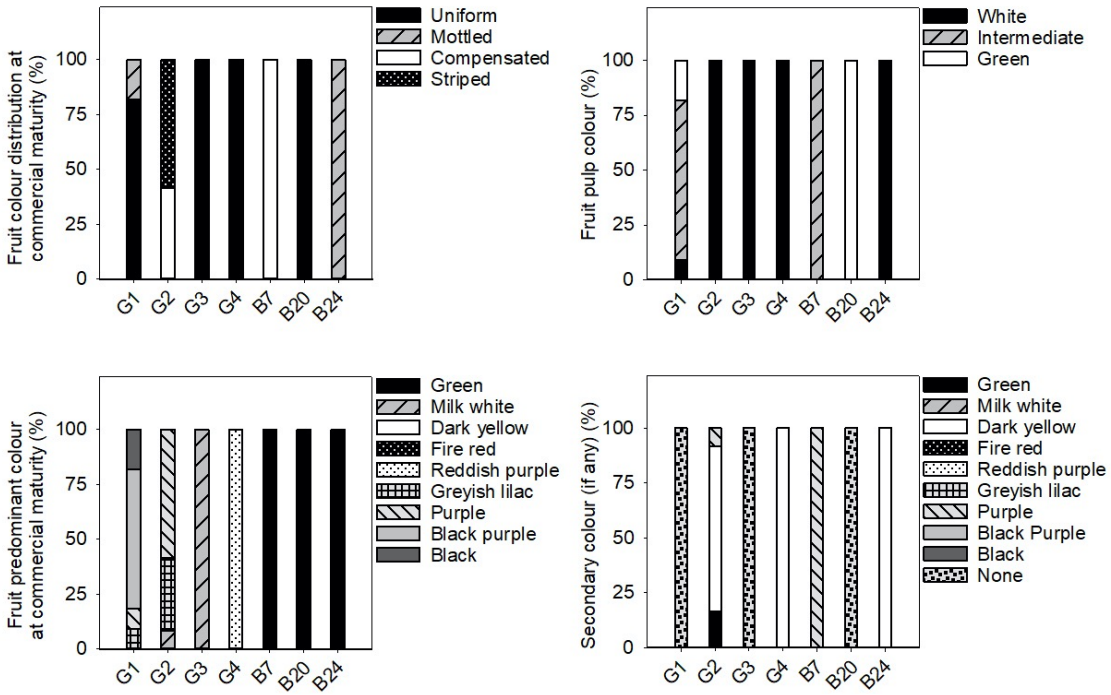
The landraces grouped for their striped fruit-skin (G2) were mostly characterised by an intermediate prostrate growth habit, medium relative internode length, large hairy leaves and pale flowers. Their fruits were elongated, non-curved with a depressed apex, white pulp, many prickles on the calyx and they were considerably heavy. Dark yellow and purple colours predominated on their skin, along with a striped or compensated colour distribution.
Three of the landraces in this study had white skin fruits (G3) and generally presented short internodes, prostrate growth habit, weak stem pilosity, absence of anthocyanins on the stem and many bright violet flowers. The afore-mentioned fruits had white pulp, many prickles, an oval-ovoidal shape and no curvature.



*** Figure 3.** Frequency distribution (%) of the fruit qualitative traits related to fruit shape and size in the 31 eggplant landraces in each group (G1, G2, G3, G4) and B7, B20 and B24. Measurements were taken when fruits reached the commercial maturity. Data for fruit traits were measured from ten different fruits which were representative of the landrace.



*** Figure 4.** Frequency distribution (%) of the stem, leaf and flower qualitative traits in the 31 eggplant landraces in each group (G1, G2, G3, G4) and B7, B20 and B24. Measurements were taken when fruits reached the commercial maturity. Data for plant, leaf and flower traits were measured from eight independent plants, which gave 8 replicates per landrace.



*** Figure 5.** Frequency distribution (%) of the fruit qualitative traits related to fruit colour in the 31 eggplant landraces in each group (G1, G2, G3, G4) and B7, B20 and B24. Measurements were taken when fruits reached the commercial maturity. Data for fruit traits were measured from ten different fruits which were representative of the landrace.

Only two accessions that produced reddish-purple fruit were detected (G4). These plants presented erect-intermediate growth habit, dense branching, short internodes, weak stem pilosity and bright violet flowers. Their fruits were elongated, ellipsoid-shaped and curved, and they presented a rounded apex, white pulp and a few prickles on the calyx.

Landraces B7, B20 and B24 were not included in any of the groups because of their distinctive fruit typology. Variety B7 had globular fruits that were equally green and purple in colour, and were very light and small in size. This landrace was also notable for its high anthocyanin content on its hairy stem. The B20 entry had very elongated ellipsoid fruits that were green in colour with no prickles on the calyx. Finally, landrace B24 had mostly green and globular-shaped fruits, but with yellow stripes on the lower part. They also characterized for their white pulp and elevated weight

3.3. Correlation among the selected agro-morphological quantitative traits

Correlation analyses were carried out to estimate the relation between the most important quantitative traits (Table 5). The pairwise coefficients showed a positive correlation and a statistical significance for 15 pairs of traits of the 55 studied ones. The most representative positive relations were observed between fruit width vs. weight, fruit ratio vs. fruit length and leaf length vs. width. Statistically significant negative correlations for pairs of traits were also determined in 6 out of the 55 studied ones. The closest negative relations were for the fruit ratio vs. fruit width and fruit length vs. calyx ratios.

Trait	Fr Length	Fr Weight	Fr Width	Lf Length	Lf Width	Nº calix pickels	Nº Fl	P Length	P Width	Fr L/W
Calyx L/ Fr L	**-0.6542 *****	0.0154	0.262	0.0743	0.0846	**0.3839 ***	**0.3658 ***	-0.2841	-0.0477	**-0.557 ****
Fr Length		0.124	-0.3517	0.1206	-0.0733	-0.0277	-0.1463	**0.4281 ***	0.0742	**0.7692 *****
Fr Weight			**0.858 *****	**0.4650 ****	**0.4088 ***	**0.4721 ****	-0.1219	-0.2166	**0.4282 ***	**-0.4887 ****
Fr Width				**0.3590 ***	**0.3974 ***	**0.4208 ***	-0.0322	**-0.4110 ***	**0.3886 ***	**-0.8218 *****
Lf Length					**0.7177 *****	0.0893	0.2532	-0.0024	0.248	-0.1921
Lf Width						0.2524	0.1997	**-0.3580 ***	0.2219	-0.2662
Nº calix pickels							0.0342	-0.3069	0.1363	-0.311
Nº Fl								-0.0549	-0.0874	-0.1027
P Length									0.3004	**0.4856 ****
P Width										-0.1965

*** Table 5.** Linear correlation coefficient (r) and its significance between the quantitative traits used for phenotyping in the collection of the 31 eggplant landraces cultivated in Spain. ***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$ for r. P, plant; Lf, leaf; Fl, flower; Fr, fruit; L/W, length/width ratio.

3.4. Nutraceutical characteristics

The fruit of three eggplant landraces (B14, B16, B17) were characterised to establish fruit quality. Significant differences ($p < 0.05$ or $p < 0.001$) were found among the average values in the selected eggplant landraces for all the analysed nutraceutical compounds, but not in the DW percentage (Table 6).

Trait	B14	B16	B17	
DW (%)	23.09 ± 4.60	21.87 ± 3.76	24.38 ± 8.53	ns
Pulp *L**	72.74 ±87.11 b	73.22-77.23 ± b	71.27 ± 88.48a	**
Pulp *a**	-5.57 ± -3.72b	-1.14 ± -1.05a	-2.27 ± -0.61a	***
Pulp *b**	17.23 ± 22.87a	9.57 ± 10.62b	7.76 ± 12.27b	***
DPPH (%)	38.29 ± 11.03a	19.75 ± 6.74b	21.71 ± 3.89b	***
Phe (*mg g^{-1}FW*)	4.47 ± 1.21a	2.53 ± 0.64b	2.61 ± 0.65b	***
Flav (*mg 100 g^{-1}FW*)	65.7 ± 23.90a	27.25 ± 11.64b	26.10 ± 8.99b	***
Asc (*mg 100 g^{-1}FW*)	10.95 ± 3.67a	4.82 ± 1.10b	12.75 ± 3.46a	***
Car (*µg g^{-1}FW*)	3.88 ± 0.17a	1.65 ± 0.21b	1.78 ± 0.16b	***
Sugars (*g 100 g^{-1}FW*)	5.34 ± 1.21b	4.84 ± 0.28b	6.48 ± 0.96a	*

*** Table 6.** Fruit quality traits in three local eggplant landraces cultivated in Spain. Values are the mean ± SD of n = 5 for dry weight and sugars, n = 20 for colour parameters and n = 10 for antioxidant traits. Different letters in a row indicate significant differences at $p < 0.05$ (LSD test). ***, **, * and ns denotes significance at $p < 0.001$, $p < 0.01$, $p < 0.05$ and non-significant values, respectively. DW: Dry Weight; L*, lightness value; a*, greenness/redness value; b*, blueness/yellowness value; DPPH: Antioxidant capacity; Phe: Phenols; Flav: Flavonoids; Asc: Ascorbic acid; Car: Carotenoid concentration

3.4.1 Fruit DW Percentage

The fruit DW (Table 6) range was 11.57 - 42.64% for the studied landraces. The mean values for cultivars B14, B16 and B17 were 24.37%, 21.87% and 23.09%, respectively, and no significant differences were observed among landraces.

3.4.2 Pulp colour

The L*, a*, b* colour parameters, measured on the fruit inner pulp, (Table 6) ranged from 71.27-88.48 (L*), -2.38 – 1.6 (a*) and 15.11 – 22.87 (b*) for the studied landraces. Focusing on colour parameter L*, significant differences were

found between landraces, turning B17 to own a lighter pulp colour (1.07-fold higher value). When analyzing colour parameter a*, it was observed that landrace B14 had a greener pulp tonality (3.62-fold higher value). Also, in accordance with b* parameter data, landrace B14, showed a much more yellowish pulp (1.97-fold higher value), what differentiated this variety from B16 and B17.

3.4.3 Nutraceutical compounds and antioxidant capacity

Antioxidant capacity

Antioxidant capacity was determined by the DPPH assay (Table 6) and its range was 9.97 - 62.65 %. Landrace B14 had a statistically higher antioxidant capacity (mean value of 38.29%) compared to B16 and B17, with no differences found between them. Antioxidant capacity was 17.56% higher (1.8-fold) in B14.

Phenols

The total phenolic content (Table 6) for the three different eggplant cultivars ranged from 1.56 to 7.48 mg g^{-1} FW. B14 obtained a significantly higher mean value for phenolic content (4.47 mg g^{-1} FW), with no differences between B16 and B17 (2.53 and 2.61 mg g^{-1} FW, respectively). Phenolic concentration was 1.7-fold higher in landrace B14, which was 42% higher than for the other varieties.

Flavonoids

The total flavonoid content (Table 6) ranged between 5.75 and 118.63 mg 100 g^{-1} FW among the three landraces. Significant differences were found in the three landraces in relation to the total flavonoid content. Landraces B16 and B17 did not show any significant differences in the flavonoid concentration, and their mean values were 27.25 and 26.1 mg 100 g^{-1} FW, respectively. B14 stood out for its high flavonoid content (mean value of 65.7 mg 100 g^{-1} FW). The flavonoid concentration was 59.4% higher in B14, which was 2.5-fold higher than B16 and B17.

Ascorbic acid

The ascorbic acid content (Table 6) range was 3.45 - 18.45 mg 100 g^{-1} FW for the three landraces. Significant differences were found in the three landraces. Landrace B16 had a statistically lower ascorbic acid content (mean value of 4.82 mg 100 g^{-1} FW) compared to B14 and B17 (mean values of 10.94 and 12.75 mg 100 g^{-1} FW, respectively), with not differences between them. The ascorbic acid concentration was 60% lower in B16, which is 2.5-fold lower than B14 and B17.

Carotenoids

The total carotenoid content (Table 6) range was 1.46 - 4.06 µg g^{-1} FW for the three landraces. The ANOVA analysis showed that landrace B14 had the highest carotenoid content (mean value of 3.88 µg g^{-1} FW). Accessions B16 and B17 did not show significant differences between them and respectively presented 1.65 and 1.78 µg g^{-1} of FW carotenoids as the mean value. The carotenoid concentration was 55.6% (2.27-fold) lower in B16 and B17 compared to B14.

3.4.4 Soluble sugars

The sugar content (Table 6) for the different eggplant cultivars ranged from 4.85 to 7.62 g 100 g^{-1} FW, which is a 1.57-fold increase in content. Considerable differences were found in the studied landraces. B17 had a significantly higher mean value for sugar content (6.48 g 100 g^{-1} FW), and no differences were reported between B14 and B16 (5.336 and 4.96 g 100 g^{-1} FW, respectively). Sugar content was 20.64% higher in B17, which is 1.25-fold increase compared to B14 and B16.

3.4.5 Correlation between antioxidant compounds

In order to estimate the contribution of the quality traits in the pulp of the fruits, several correlation analyses were carried between the different combinations of the percentage of DW, colour, nutraceutical compounds and sugar data (Table 7). The pairwise coefficients showed a positive correlation and a statistical significance for eleven pairs and only two negative correlations. While DW was not correlated with any trait, the colour parameters in the pulp showed marked correlations. The strongest and most positive ones were registered between b* value and four of the five nutraceutical compounds (r = 0.9604 for carotenoids and r = 0.5769 - 0.6327 for DPPH, phenols and flavonoids). By contrast, a strong but negative correlation was observed between a* and carotenoids content (r = -0.9771) while it was moderate and positive between L* and sugar content (r = 0.6226). When comparing nutraceutical compounds, four strong significant and positive correlations were recorded between the combinations of DPPH vs. phenolics, DPPH vs. flavonoids, DPPH vs. carotenoids, and phenolics and flavonoids, where the coefficient r ranged from 0.7955 to 0.8322. Phenols vs. carotenoidsalso showed moderate and positive correlation (r = 0.6898). Related to sugar content, it was positively correlated with carotenoids content (r = 0.6302).

Trait	Pulp *L**	Pulp *a**	Pulp *b**	DPPH	Phe	Flav	Asc	Car	Sugars
% DW	-0.2284	0.007	-0.03	-0.0694	-0.0077	-0.0203	-0.0677	-0.0969	-0.1582
Pulp *L**		-0.2432	**-0.3297 ***	-0.136	-0.145	-0.1223	0.2949	0.2295	**0.6226 ***
Pulp *a**			0.0245	-0.0267	0.0202	-0.0087	-0.3182	**-0.9771 *****	0.088
Pulp *b**				**0.6327 *****	**0.5864 *****	**0.5769 *****	0.3631	**0.9604 *****	-0.1974
DPPH					**0.8263 *****	**0.8322 *****	0.1716	**0.7955 ****	-0.2803
Phe						**0.8256 *****	0.1461	**0.6898 ***	-0.3188
Flav							0.1289	0.4348	-0.3722
Asc								0.5248	**0.6302 ***
Car									0.2121

*** Table 7.** Linear correlation coefficient (r) and its significance between fruit quality traits (dry weight, pulp colour, nutraceutical compounds and sugars) in the collection of the three eggplant landraces (B14, B16, B17) cultivated in Spain. . ***, **, * indicate significant at p < 0.001, p < 0.01, p < 0.05 values for r. DW: Dry Weight; L*, lightness value; a*, greenness/redness value; b*, blueness/yellowness value; DPPH: Antioxidant capacity; Phe: Phenols; Flav: Flavonoids; Asc: Ascorbic acid; Car: Carotenoid concentration.

4. DISCUSSION

The morphological diversity of eggplant landraces has been the subject of many studies [49–55]. These surveys are necessary since they provide germplasm banks with very useful information, and they contribute to optimise plant breeding programmes. According to Uddin et al. [56], clustering accessions in different groups may be useful for providing a basis for further crop improvement. Many characterisation studies based on standardised morphological and agronomic descriptors developed by the International Board for Plant Genetic Resources have been performed in eggplants, and have demonstrated that they are suitable for providing very helpful information for eggplant breeders [50,53,57]. In view of the success of these surveys, the characterisation of the selected valencian varieties was made following IBPGR guidelines. Furthermore, nutraceutical quality also defines a relevant role in crop improvement [58], mainly due to eggplants' antioxidant content [59], including polyphenols, ascorbic acid and carotenoids [45], among others.

The PCA has been previously used to determine the most important traits for landrace characterisation of different species, such as sweet potato [60], spider plant [61], African tomato landraces [62] and eggplant [56,63,64]. According to our results, when subjecting the phenotypic data of the 31 landraces to the PCA analysis, nine principal components were established and corresponded to an 80% total variation. Of the nine components, none explained more than 25% of the diversity among landraces. For this reason, from the PCA analysis we inferred a wide diversity among accessions, even if landraces belonged to the same Mediterranean area. Muñoz-Falcón et al. [52] suggested that local conditions, in addition to the selection processes followed by farmers, generated a differentiation in the eggplants of the same origin. Likewise, together with this diversification process, as eggplants are generally self-pollinated plants [65], the genetic isolation of various eggplant populations may has been favoured. The mayor principal component that explained 23.3% of the total variability correlated mainly with the fruit descriptors. This separation of accessions associated with fruit traits has also been described by other authors [50,54,64], which confirms that the morphological variation in the organ for which a crop is selected widens during the domestication process [66]. Despite the genetic bottleneck that eggplant domestication has undergone [67], considerable diversity is found among landraces, unlike that seen in commercial varieties, especially in F1 hybrids [53]. Although commercial hybrids have been selected for traits like earliness, yield, lack of prickles or colour, the diversity of other morphological characters has been narrowed [50].

The correlation analysis measures the degree of relation between the selected phenotypic quantitative traits, distinguishing remarkable characters for crop improvement [68,69]. Of the obtained values, two clear trends were observed. The higher the plant height value is, the longer and lighter fruits are, while the wider the plant is, the wider and heavier its fruits. Leaf length and width were positively and significantly correlated with the average fruit weight ($r = 0.4650$ and $r = 0.4088$) and width ($r = 0.359$ and $r = 0.3974$). A larger foliar area can offer better accumulation of photosynthates in plants, to ultimately produce heavier and larger fruits [69]. A statistically significant relation existed between the number of calyx prickles with fruit weight ($r = 0.4721$) and width ($r = 0.4208$), while the absence of calyx prickles is desirable for harvest processes or consumer handling. On the contrary, a negative, but statistically significant, correlation occurred between the calyx length ratio and fruit length ($r = -0.6542$). Owning a short calyx (~ 20%) is a desirable attribute from the phytosanitary point of view since it helps to prevent eggplants from white

mites, Botrytis cinerea and several fungal diseases, whose presence is favoured by relative high humidity as petals are adhered between the calyx and fruit [70]. On this matter, and in relation to this trait, slightly heavy and elongated fruits would be preferable. Flower number per inflorescence was positively correlated with the calyx length ratio (r = 0.3658), while showing a negative tendency toward a relation with big and heavy fruit. Altogether would mean that plants with great number of flowers, which are also able to develop many eggplants per plant, would develop small calibre fruits. This tendency has also been described by other authors in eggplants [63,64]. In general terms, pubescence on leaves, lack of prickles on leaves and the calyx, erect growth habit and long fruit development are desirable attributes that facilitate the agronomic work of eggplant crops, especially concerning the harvest. They also meet the needs of both producers and final consumers [70].

Regarding fruit pulp quality, our study established significant differences in landraces B14, B16 and B17 as the antioxidant capacity of B14 was 17.56% higher than in the other cultivars. Other authors have also reported wide variability in antioxidant capacity in eggplant landraces [9,17,71,72]. Total antioxidants levels have been widely established in eggplant, but very little attention has been paid to their distribution within the fruit and their stability in different genotypes [26]. To our knowledge, very few studies claim that the major antioxidant capacity is found in fruit pulp [73]. Likewise from the information obtained from this minority of studies, it is known that the inner or central part of pulp has the greatest antioxidant [41], for this reason our results could seem exaggerated compared to studies which have worked with whole fruits.

Among vegetables, eggplants are an important source of phenolics, flavonoids and ascorbic acid, all of which are powerful antioxidants [74]. Phenolics in eggplant have been identified as the major bioactive compounds responsible for their antioxidant effects [75] and genotypes are highly diverse in the proportions of these compounds [26]. This statement agrees with the results herein obtained, where landrace B14 stood out for its high phenolic content (4.47 mg g^{-1} FW), which was 42% higher than in B16 (2.53 mg g^{-1} FW) and in B17 (2.61 mg g^{-1} FW). These results are higher than the values reported by several authors [9,12,76–78], but are similar to those obtained by Plazas et al. [79] and Niño-Medina et al. [71]. Also, when comparing these eggplants with other crops, we found that eggplant showed higher values than most of the vegetables and just few species as green pepper (2.47 mg g^{-1} FW) [80], spinach (2.69 mg g^{-1} FW) and red onion (2.53-3.11 mg g^{-1} FW) reached similar levels. Moreover, even if the phenolic content in eggplant is comparable to that found in many types of fruits, such as in strawberry (3.64 mg g^{-1} FW) [81], plum (3.04 mg g^{-1} FW) blueberry (4.25 mg g^{-1} FW) and blackberry (2.47 mg g^{-1} FW) [82], significantly exceeds the phenolic content of many others as apples (around 1 mg g^{-1} FW), sweet cherries (7.88 mg g^{-1} FW), raspberries (1.79 mg g^{-1} FW) or black grapes (2.13 mg g^{-1} FW) [80].

Significant differences were detected when determining the total flavonoid concentration of the three selected landraces. The flavonoid concentration was 59% higher in landrace B14 (65.7 mg 100 g-1 FW), while it came close to 26 mg 100 g-1 FW in landraces B16 and B17. However, other studies performed in different eggplant landraces unseat our candidates because they had much higher flavonoid concentrations; 1.733 mg 100 g-1 FW [83], 1.991–3.954 mg 100 g-1 FW [25], 370 mg 100 g-1 FW [84], 142.16–718 mg 100 g-1 FW [19], 152.4–392 mg 100 g-1 FW [85]. Nevertheless, other authors have presented comparable results to ours [21,76,86], and even much lower ones [9,87,88]. All together, these works suggest a very wide diversity among eggplant landraces and/or crop management, climate

conditions, etc. Although the obtained results on our varieties for flavonoids were not striking, it must be remembered that the key lies in the wide variability among traditional varieties, and in the need to account for several bioactive compounds to determine the whole antioxidant capacity of a given variety. Likewise, the present results are comparable to those obtained in other vegetables like beetroot (62.8 mg 100 g-1 FW), red onion (36–56 mg 100 g-1 FW) [81] and carrot (26.7 mg 100 g-1 FW) [80], but higher than those expected in pepper (7–11 mg 100 g-1 FW) [81] or tomato (4–26 mg 100 g-1 FW) [89]. However, the flavonoid content of eggplant is much lower compared to that of leafy vegetables, for example, spinach (133.1 mg 100 g-1 FW) [81] and lettuce (97.2 mg 100 g-1 FW) [80]. When making this same comparison with different fruits, we observed that the amount of flavonoids found in most of them falls within the range of our results, with some exceptions; plum (136.2 mg 100 g-1 FW), dogwood berry (91.4 mg 100 g-1 FW) [80], and mulberry (250.1 mg 100 g-1 FW) [81].

Other non-destructive methods, using remote optical methods like hyperspectral analysis and multispectral fluorescence, are also suitable for predicting the right content of some polyphenols with a good correlation [35]. Therefore, non-invasive methods enable a rapid pre-screening (positive or negative) of tens or several hundred thousands of individuals from which the best samples will be selected and tested by metabolomic analyses, which will greatly increase the efficiency of the entire process [36] and allow monitoring the evolution of these compounds along the growth cycle. However, the use of these techniques still needs to be perfected and facilitated.

The significant differences found across accession in vitamin C content suggest genotype dependence for this trait in eggplant. The average ascorbic acid concentration in the different landraces ranged from 3.46 to 18.47 mg 100 g-1 FW. These values are consistent with several studies carried out on eggplant landraces [90,91]. Some studies, such as Prohens et al. [77], have even found major differences between traditional varieties and commercial hybrids, meaning that, on average, landraces present higher ascorbic acid content. Although many foods contain a similar vitamin C content, such as onion, pineapple [92], blackthorn [82] and apple [93], the ascorbic content of many other popular fruit and vegetables, such as orange, kiwi, grapefruit, strawberry [92], blueberry [82], pepper and date [93], is much higher than that found in eggplants. Ascorbic acid is a potent antioxidant, so the relatively low ascorbic acid content in eggplant fruits may limit the whole plant's antioxidant capacity [12]. Nevertheless, this deficiency may be balanced out with its high phenolic content.

The antioxidant activity of carotenoids and their biochemical properties that influence disease prevention have also been discussed [94]. The average total carotenoid concentration in the eggplant landraces ranged from 1.46 to 4.06 µg g-1 FW, which gave a mean value of 2.5 µg g-1 FW. Very few studies have been carried out on eggplants in which total carotenoids content ranged from 0.44 to 1.22 µg g -1 FW [95] or obtained 1.32 µg g-1 FW as the mean value [96]. According to the National Nutrient Databases for Standards Reference (USDA), the mean carotenoid content in eggplants is 0.16 µg g -1 FW. Although, the local varieties herein used appear to have a much higher carotenoid content on average, in other crops as in zucchinis (28.34 µg g-1 FW) [96], and green peppers (20 µg g-1 FW) [97]. These concentrations are more than eight times as much compared to the carotenoid content in eggplant, but are almost worthless compared to red peppers (130 µg g-1 FW) [97] and carrots (95.93 µg g-1 FW) [96].
The correlations found in this study between antioxidants, fall in line with those presented by Barreto et al. [98], Ramaiya et al. [99], and Fratianni et al. [100], who suggested that antioxidant capacity was positively linked with the

amount of total polyphenols, and was less related to the content of both carotenes and ascorbic acid. In this study, statistically remarkable relationships were found between phenolics and carotenoids (r = 0.7955), but not between ascorbic acid and phenols. Andarwulan et al. [101] stated that ascorbic acid is known to contribute to total phenolic content, even when no correlation is observed between them. A positive relationship between these two parameters was detected by Hanson et al. [28] in tomato, which supports this idea. Finally, a moderate and statistically significant correlation was observed between ascorbic acid and sugar content (r = 0.6302). This same relationship was detected by Ramaiya et al. [99] in papaya, associating it to the common and complex interactions existing between organic acids and sugars.

Some correlations between nutraceuticals and colorimetric parameters in the pulp were also registered in the experiment. A moderate correlation was detected between the L * parameter and the sugar content of the pulp, a relationship also highlighted by Orak [102] in red grapes pulp. It is known that eggplant fruits accumulate sugars preferentially in the inner pulp [103]. Therefore, in our case, as the colorimetric data was taken in this particular region of the pulp, the concentration of sugars may have modified the value of L * . In addition, parameter b * was related to antioxidants as a general rule, a fact also mentioned by Orak [102]. Finally, a * appears to be negatively related to carotenoids, which is to be expected since this parameter detects reddish shades and carotenoids are known to provide from yellowish to reddish tones [104].

Health-conscious consumers generally focus on the antioxidant capacity, and the phenolic and vitamin contents of foods [105]. However, fruit quality is determined primarily by taste, and a major component of taste is sugar content [106]. Hence, its analysis is recommended in eggplant, as fruits are believed to be rich in this compound and could therefore satisfy consumers [107]. Even though significant differences were found among the three landraces analysed in this assay, and landrace B17 stood out for its high sugar content, compared to data from other studies on eggplants [78,90,103,108–110], it is observed that sugar concentration is almost doubled in our landraces.

5. CONCLUSIONS

The herein reported results showed the high degree of diversity among the selected traditional eggplant varieties. Among morphological characteristics that may be of interest for handling jobs like crop harvesting, are included having an erect growth habit, low branch density, lack of hairiness on leaves, no prickles on the calyx and the development of elongated and not excessively heavy fruits. Between groups G1 and G2, which include similar varieties to those marketed today, landraces B4, B12 and B19 could be highlighted based on the previous traits. Trade in the G3 and G4 varieties could also be promoted because: white-fruited varieties (G3) produce many flowers and somewhat elongated fruits of an attractive colour for consumers, while the reddish fruit entries (G4) produce elongated but not too heavy fruits, with a few thorns on the calyx, which could be interesting options. As the nutritional profile is helpful for promoting the commercialisation and consumption of local varieties, and according to the nutritional quality part of this study, variety B14 could be promising for human consumption, mainly for its antioxidant properties. Taken together, this information could be relevant for future plant breeding programme to obtain easily manageable and harvestable eggplant varieties.

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CHAPTER 06

THE NUTRITIONAL QUALITY POTENTIAL OF MICROGREENS, BABY LEAVES AND ADULT LETTUCE; AN UNDEREXPLOITED NUTRACEUTICAL SOURCE

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Abstract

Abstract: Interest in the cultivation of lettuce landraces is increasing because native varieties particularly attract consumers as high quality products. Lettuce is a popular leafy vegetable worldwide, and interest in the consumption of first leaves (microgreens) and seedlings (baby leaves) has grown due to the general belief of young plants offering higher nutritional value. The content of some bioactive compounds and antioxidants (chlorophylls, carotenoids, anthocyanins, ascorbic acid, phenols, antioxidant activity) was monitored in six lettuce landraces and five commercial varieties, and compared in three development stages: microgreen, baby and adult.Ascorbic acid and phenolic contents were 42% and 79% higher, respectively, in early stages than in adult lettuces, and red-leaf varieties (CL4 and L11) stood out. This finding agrees with their marked antioxidant capacity and correlates with pigment contents, especially anthocyanins. The nutritional value of adult lettuce is conditioned by its size, shape and head structure as phytochemical concentrations are regulated by light. The low content of ascorbic acid, phenolics and anthocyaninsin crisphead lettuce (CL5) is a clear example (49, 67% and 27% lower, respectively, than adult mean).

Our results indicate the wide variability in lettuces for nutritional characteristics and emphasizes that traditional varieties are a helpful source of agricultural biodiversity.

Keywords:

Antioxidant, biodiversity, baby leaf, landrace, lettuce, microgreen, mineral, nutraceutical compound

1. INTRODUCTION

Lettuce ***(Lactuca sativa L.)*** is a popular and widely grown leafy vegetable worldwide, currently in salad mixes whose consumption is increasing. Lettuce can contribute significantly to the nutritional content of diets [1]. In recent years, general consumer and researcher concern has been voiced about foods which, beyond nutritional needs, also provide health-beneficial effects; for example, they promote well-being, reduce diseases and prolong life span. All this is related to the nutritional quality of vegetables (minerals, vitamins, and phytochemicals of considerable antioxidant potential) [2,3].

The biosynthesis, composition and concentration of health-promoting compounds widely vary among leafy vegetables, and carry the influence of genetic and environmental factors, growing conditions, harvest practices and postharvest handling conditions [4]. As lettuce is generally eaten raw, more nutrients are preserved than in other cooked or processed vegetables like potatoes. Nevertheless, lettuce has not been regarded as a nutritional food, primarily because of its high water content (around 95%), but its nutrient composition may be the equivalent to other vegetables [5]. In lettuce, different plant attributes, such as leaf colour, may influence nutritional quality. One clear example is leaf pigmentation, which is often associated with the presence of antioxidant compounds. Red lettuce is highlighted for its lipophilic antioxidant activity, ascorbic acid and phenolic contents compared to other leafy vegetables (chicory, green lettuce, lamb's lettuce, mizuna, red chard, red lettuce, rocket, spinach, Swiss chard, tatsoi), especially when exposed to low photosynthetically active radiation (PAR) light intensity. High phenolic compounds have also been observed for green lettuce at high PAR [6]. In 11 lettuce cultivars, Lata and Przeradzka [7] determined that the antioxidant capacity provided by glutamic acid and ascorbic acid contents was higher in the cultivars Kobra, Marion and Red Bowl. Gazula et al. [8] worked with nine lettuce cultivars with differing numbers of genes to regulate carotene synthesis in them, and found that the highest pigment concentrations were in the cultivars with the most genes in question. Comparisons of lettuce mineral contents are limited by the wide variation in the mineral contents reported in studies. This may be due to factors like different soil mineral compositions [9] and lettuce head types [10]. Studies generally report lettuce to offer a relatively good source of Fe and little Na. Overall among plant types, mineral content was higher in butterhead, romaine and leaf lettuces than in crisphead (iceberg) [11]. As lettuce is characterised by its marked ability to accumulate nitrate in leaves, its low concentration is considered one of the most important healthy parameters that is influenced by both genetic and environmental factors, especially light intensity [12].

Finally, plant age is interesting given the general belief that young plants have a higher nutritional value [13]. Eating the first-development leaves (microgreens) to add texture and flavour to various dishes and salads consisting of seedlings (baby leaves) has gained popularity as a culinary trend [14,15]. This trend has been driven by two important market chain memberships: 1) growers, whose marketing strategy to obtain higher profits seeks to diversify the offer and reduce cultivation periods; 2) consumers, who constantly search for potential nutritional food and can make the most of their easy home cultivation, especially as their availability in shops is scarce. So given the popularity of lettuce worldwide, microgreens and baby types constitute a novel functional food that combines high sensory and bioactive values. This inspires making comparisons to their mature-leaf counterparts, particularly as very few studies have examined their vitamin, nutrient and carotenoid contents [9,15] and even fewer have provided comparative evidence for the phytochemical content of microgreens and baby leaves as opposed to their mature-leaf counterparts. The

studies of Pinto et al. [9] and Weber [16] address solely the comparative mineral profiles between mature leaves and microgreens. El-Nakhe et al. [17] compares some nutraceutical compounds (Chlorophylls, vitamin C, carotenes, phenolics), but this study was carried out with only two lettuce varieties at two harvest times (microgreen and adult).

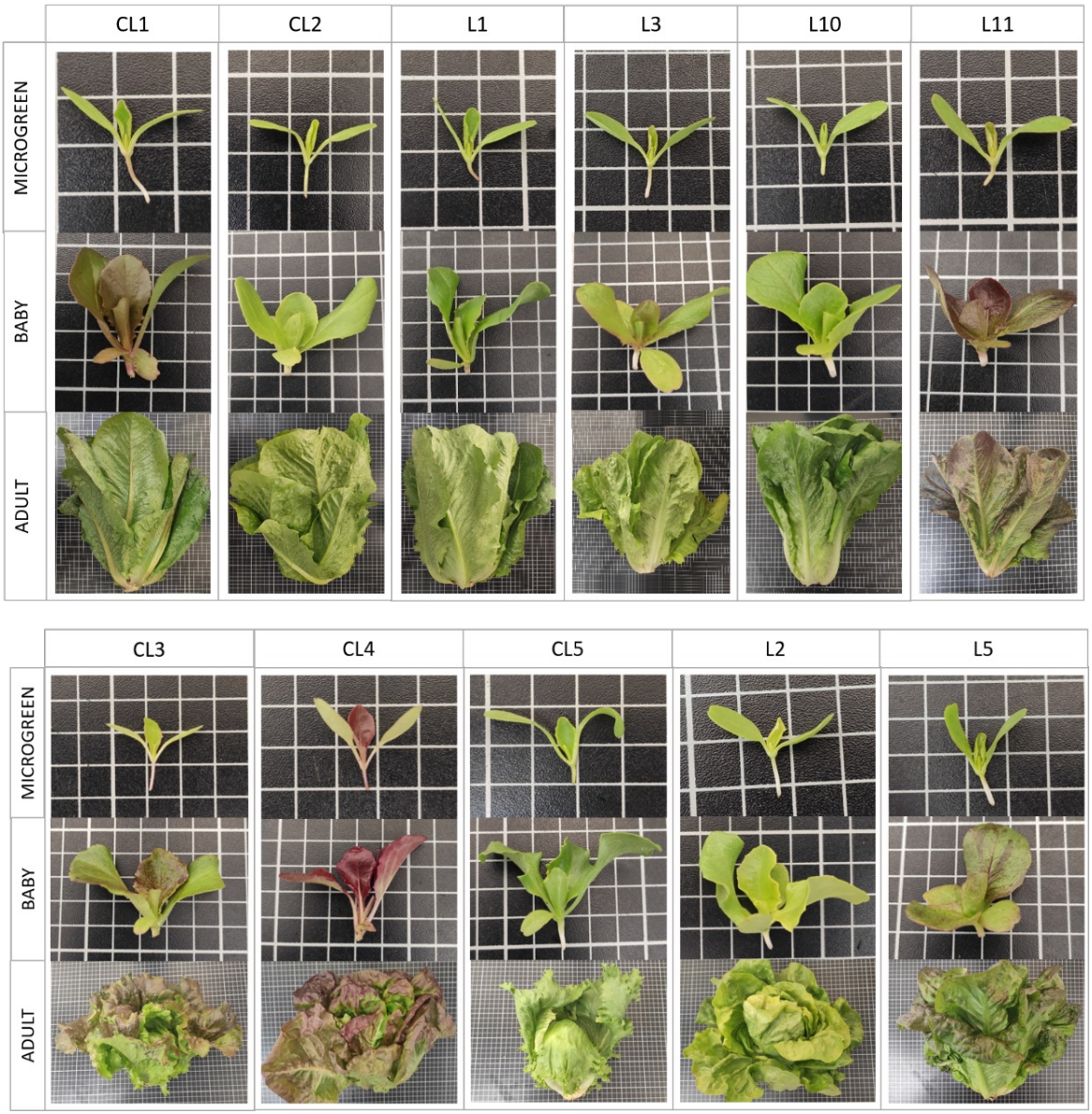
Another factor to induce variations in nutritional quality in lettuce is genetic material. Although there is compelling evidence for a declining nutritional value of horticultural crops, which is attributed to both changes in agricultural practices and the replacement of landraces with modern varieties and hybrids, promising new sources of diet lie in local landraces, underutilised crops and edible wild plants [18].

Hence, this work aims to report the nutritional value of lettuce in relation to its different morphologies (colour and head structure) and three harvest stages (microgreens, baby leaves and adults) to determine the best health-beneficial candidates that provide the highest nutritional value and bioactive compounds. Finally, it compares six Valencian lettuce landraces and five similar commercial varieties, as well as values like the usefulness of local varieties as a source of biodiversity.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material for this study consisted of 11 lettuce ***(Lactuca sativa L.)*** varieties, including six landraces from the Valencian Community (Spain), which represent diversity in leaf colour and head morphology. Seeds were provided by the Valencian Institute for the Conservation and Improvement of Agrobiodiversity (COMAV-UPV, Spain) and the Valencian Institute of Agrarian Research (IVIA, Spain). In addition, five commercial varieties were chosen as the most representative of market formats. Table 1 provides the technical information of each variety. Figures 1A and 1B complement this table.



*** Figure 1.** Pictures of the 11 cultivated lettuce varieties ***(Lactuca sativa L.)*** in the three development stages (microgreen, baby, adult) provided by the Germplasm Banks from the COMAV and the IVIA (Spain). The size of the grid cells in the fruit pictures is 1 cm × 1 cm. A: lettuce varieties without a patent head; B: lettuce varieties with a prominent head

Abbreviation code	Origin	Identification	Plant description
CL1	Commercial	Romaine lettuce long mule ear (Battle)[a]	Dark green. Elongated shape. Compact and narrow head, barely prominent.
CL2	Commercial	Romaine lettuce from the gardeners (Vilmorín)[a]	Green-yellowish. Elongated shape. Compact and narrow head, barely prominent.
CL3	Commercial	Wonder summer (Battle)[a]	Green with reddish shades. Remarkable width in relation to height. Compact, rounded and quite prominent head.
CL4	Commercial	Marvel of Four Seasons Butterhead (Battle)[a]	Dark green with reddish red is prominent at the edges. Round shape. Quite rounded shape. Full-size head.
CL5	Commercial	Batavia, iceberg type (Battle)[a]	Not very intense green. Rounded shape. Full-size head..
L1	Local landrace	BGV5721[b]	Dark green. Pink shades near the principal stem. Elongated shape. Compact and narrow head, barely prominent.
L2	Local landrace	BGV5722[b]	Green-yellowish. Rpund shape. Full-size head.
L3	Local landrace	BGV5723[b]	Green-yellowish. Remarkable width in relation to height. Head not appreciated.
L5	Local landrace	BGV5736[b]	Dark green with reddish shades. Elongated shape. Compact and narrow head, quite prominent.
L10	Local landrace	L-10[b]	Dark green. Elongated shape. Compact and narrow head, barely prominent.
L11	Local landrace	L-11[b]	Dark red, almost purple. Remarkable width in relation to height. Head not appreciated.

*** Table 1.** Abbreviation, origin, identification and short phenotypic description of the 11 lettuce varieties used in the study. The plant material from local landraces was provided by the: (1) Valencian Institute for the Conservation and Improvement of Agro-biodiversity (COMAV, Spain); (2) Valencian Institute for Agricultural Research (IVIA, Spain).
[a]Commercial name (company), [b]Genbank code

2.2. Greenhouse-field experiments

Experiments were conducted from November to March in the IVIA experimental installations in Moncada (Valencia, Spain; 39° 35′ 22.3′′ N, 0° 23′ 44.0′′ W, 37 cm above sea level). Seeds were sown in November 2020 in 104-hole trays with 100% natural coconut coir fibre substrate (225 g L-1 density, Cocopeat, Projar Co., 46930 Quart de Poblet, Valencia, Spain) under greenhouse conditions (natural light conditions with a maximum PAR of 1,000 µmol m^{-2} s^{-1}, a mean temperature of 21°C and a mean humidity of 60%).

Two weeks after germination, the first group of seedlings (microgreen stage) was collected, after ensuring that the first true leaf had appeared, by cutting seedlings at the substrate level. Each microgreen sample was formed by at least 20 seedlings, and each landrace or commercial variety consisted in four replicates.
A second group of plants was moved to an unheated greenhouse, where temperatures and light incidence were the same as in the external environment to, thus, prevent seedling thinning caused by high temperatures. Seedlings were protected from wind and potential pests while growing until the majority of plants were 5 cm high (around 4 weeks after germination) after ensuring that at least four true leaves had appeared. One subgroup of seedlings was collected (baby stage) by cutting seedlings at the substrate level. Each landrace or commercial variety consisted in four replicates formed by at least 10 seedlings each. The average range of the minimum and maximum temperatures was 4–26°C for November and -1–26°C for December.

Finally, a second subgroup of seedlings was transplanted on 4 December 2020, and grown under field conditions. Each landrace or commercial variety consisted in 20 plants grown in two separate replicates (10 single plants each) cultivated in single rows (110 cm apart) with 30-centimeter and 60-centimeter spacing between each plant and variety, respectively. The plot was surrounded by border rows on all four sides. The soil composition within a depth of 20 cm was 68% sand, 11% clay and 21% silt (sandy-clay loam), and contained 0.61% organic matter, 0.051% total N, less than 8 mg kg−1 of P, 301 mg kg−1 of K and 2.87 meq·100 g−1 of assimilable Mg. Soil electrical conductivity was 0.290 dS m−1 and pH was 8.1.

Irrigation satisfied 100% crop evapotranspiration (ETc), as described in Penella et al. [19] performed with a drip system. Nutrients were applied by the irrigation system at a rate (kg ha-1) of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO and 35 MgO, as recommended by Maroto [20]. The average range of the minimum and maximum temperatures during the field experiment was 1–23°C for December, -1–26°C for January, 7–24°C for February and 6–26°C for March. Plants (adult stage) were harvested on 16 March.

2.3. Leaf sample preparation

For the microgreens and baby material, eight different replicates (2 g of vegetal material per replicate) of each variety were obtained by randomly grouping seedlings (around 20 and 10 plants each replicate for microgreen and baby, respectively). Four of these replicates were reserved for stove drying. The remaining fresh samples were instantly frozen in liquid nitrogen and stored at -80°C. Of the adult plants, four different replicates (1 individual lettuce each replicate) were harvested from the field. Lettuce was cut lengthwise into four halves. A fraction of each lettuce was set aside for drying. A second fraction was chopped and instantly frozen in liquid nitrogen and stored at -80ºC.

The plant material reserved for drying was used for the mineral analysis and dry weight (DW) quantification, while the samples stored at -80°C were employed for nutraceutical quality determinations. Samples were ground in a mixer mill (MM400, Retsch, Hann, Germany) with liquid nitrogen to prevent melting. The same machine was used to homogenise the samples dried in a laboratory oven at 65°C for 72 h.

2.4. Nutraceutical compounds and antioxidant capacity

2.4.1 Chlorophyll and carotenoid concentration

Total chlorophyll (Chl) a+b and carotenoids (Car) concentration were determined spectrophotometrically as described by Porra et al. [21]. Briefly, 2.5 mL of 80% acetone (v/v) were added to sample extracts (0.06 g FW) and centrifuged at 2,000 rpm for 8 min. The supernatant was used for the analysis. Solution absorption was measured at 663.6, 646.6 and 470 nm in a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). Then 80% acetone (v/v) was utilised as the blank solution. The chlorophyll and carotenoid contents of extracts were calculated by the following equations:

(1) Chl a = 12.25 × Abs663.6 − 2.79 × Abs646.6 (µg mL−1)
(2) Chl b = 21.3 × Abs646.6 − 5.1 × Abs663.6 (µg mL−1)
(3) Car = [(1000 × Abs470 − 1.82 Chl a) − (85.02 × Chl b)]/198 (µg mL−1)
(4) Chl a + b = 7.15 x Abs663.6 + 18.71 x 646.6

Chlorophylls and carotenoids were expressed as µg g−1 FW.

2.4.2 Anthocyanin concentration

The anthocyanin (Ant) concentration was spectrophotometrically quantified as described by Szepesi et al. [22]. Five mL of methanol:HCl:H_2O solution (90:1:9) were added to 0.1g of FW of the homogenised sample previously placed in glass tubes. Samples were vortexed and stored in the dark for 1 h. Those in tubes were mixed at room temperature. Then they were centrifuged at 2,000 rpm for 5 min and the supernatant was used for the analysis. Solution absorption was measured at 534, 643 and 661 nm in a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). Methanol:HCl:H_2O solution was employed as the blank. The Ant content of the extracts was calculated by the following equation:

(1) (0.0821 x Abs534 − 0.00687 x Abs643 − 0.002426 x Abs661) * 5 mL g^{-1} FW

The anthocyanin concentration was expressed as µmol 100g-1 FW.

2.4.3 Ascorbic acid concentration

The total ascorbic acid (AsA) content was spectrophotometrically quantified as described by Kampfenkel et al. [23]. First 0.2 g FW of each homogenised sample were added with 1.5 mL of 6% (w/v) trichloroacetic acid (TCA). Samples were centrifuged at 15,000 rpm for 5 min at 4ºC and the supernatant was recovered. Then 0.05 mL of the homogenate was mixed with 0.05 mL of 10 mM dithiothreitol (DTT) and 0.1 mL of 0.2 M phosphate buffer (pH 7.4). Samples

were incubated for 15 min at 42°C. Next 0.05 mL of 0.5% (w/v) N-ethylamide (NEM) were added and incubated for 1 min at room temperature. Afterwards 0.25 mL of 10% (w/v) TCA, 0.2 mL of H_3PO_4 4% (w/v) and 0.2 mL of 2-2′-dipyridyl and 0.1 mL of 3% (w/v) $FeCl_3$ were added to the solution. They were incubated together in a water bath for 40 min at 42°C. Solution absorption was measured at 525 nm in a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The blank solution with no extract was used for calibration. AsA was expressed as mg 100g-1 FW.

2.4.4 Total phenolic analysis

Phenolic (Phe) content was analysed according to Dewanto et al. [24] with minor changes. Firstly, a 0.1 g FW aliquot of the homogenised sample was homogenised in 0.7 mL of 80% (v/v) methanol, vortexed, incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min, and then revortexed. Samples were centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was reserved. The total Phe content was determined by the Folin-Ciocalteau colorimetric method. Following this order, a 20 µL aliquot of the supernatant was mixed with 80 µL of methanol and 0.7 mL of Folin-Ciocalteau reagent. This solution was vortexed and incubated in the dark for 5 minutes at room temperature. Next 0.7 mL of $NaHCO_3$ (6%) were added. The final solution was vortexed and incubated in the dark for 60 min. Solution absorption was measured at 765 nm in a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The blank solution with no extract was used for calibration. Each measurement was compared to a standard curve of gallic acid (GA). The Phe concentration was expressed as mg of GA equivalent g-1 FW.

2.4.5 Antioxidant capacity measurements

Antioxidant capacity (DPPH) was measured following the method reported by Brand-Williams et al. [25] with minor changes. Firstly, 0.1 g FW of sample was homogenised in 0.7 mL of 80% methanol (v/v), incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min and then vortexed. Samples were centrifuged at 10,000 rpm for 15 min at 4°C and 20 µL of the extract were added to 990 µL of 0.065 M of 2,2-diphenyl-1-picrylhydrazyl solution (solved in 80% methanol). Absorbance was measured at 515 nm against a blank solution (80% methanol without extract) after a 30-minute reaction time at room temperature in the dark in a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The results were expressed as the percentage reduction of the initial DPPH absorption in extracts.

2.5. Mineral determination

Samples were dried in a laboratory oven at 65°C for 72 h and homogenised before being burnt in a muffle furnace for 12 h at 550°C. Macronutrients and micronutrients were extracted with 5 mL of 2% (v/v) nitric acid in an ultrasonic bath for 30 min at 40ºC. Afterwards 10 mL of 2% nitric acid were added to the solution. Mineral concentrations were measured by ICP emission spectrometry (iCAP 6000, Thermo Scientific, Cambridge, UK). The results for the

macro- and micronutrients were expressed as mg g-1 DW and µg g-1 DW, respectively.

2.6. Statistical analysis

The results obtained from these determinations were subjected to a one-way analysis of variance (ANOVA) using Statgraphics Centurion XVII (Statistical Graphics Corporation 2014). The statistical analysis was carried out after taking two different factors into account; variety type and development stage. The results were expressed as mean±standard deviation. Means were accepted as being significantly different at a 95% confidence interval ($p \leq 0.05$). The mean, maximum and minimum values, coefficient of variation, and F-ratio of all the traits were calculated.

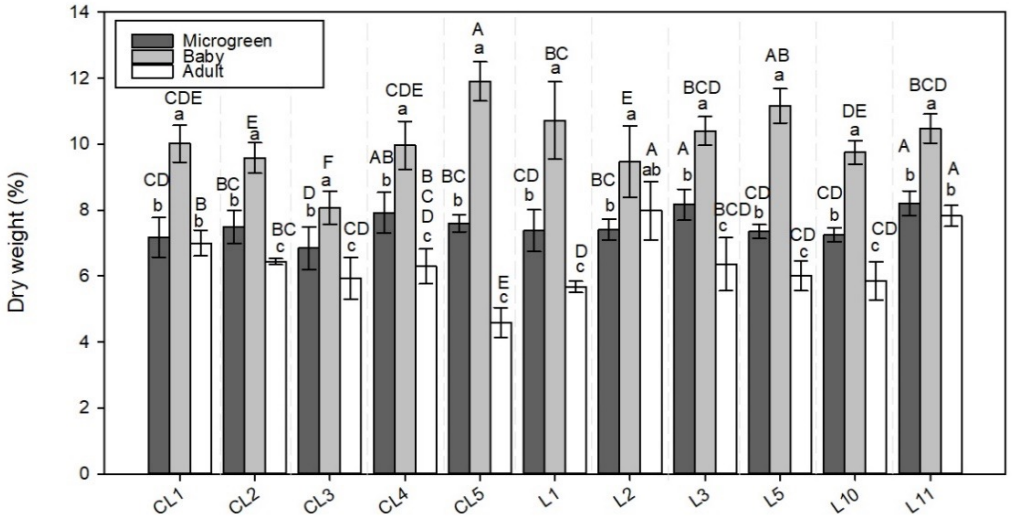
A principal component analysis (PCA) was run for the standardised values using pairwise Euclidean distances among accession means to determine the relations between genotypes in each development stage. The extracted eigenvalues, and the relative and cumulative proportions of total variance explained by the first three principal components (PCs), were calculated. A two-dimensional (2D) scatter plot (first PC vs. second PC) for each development stage was prepared based on a distance matrix for the PCs to visualise the relation that explained traits.

By considering quality traits, three correlation analyses were completed among varieties, one for each development stage. The individual samples of each accession were subjected to linear regression and correlation coefficients (r) were obtained.

3. RESULTS

3.1. Dry weight

Three varieties (CL1, L2, L11) presented no statistical differences in % DW between the microgreen and adult plants (Fig. 2). The highest values were recorded for CL4, L3 and L11 in the microgreen plants (nearly 0.7% higher than the mean value) and CL5 and L5 in the baby stage (1.8 and 1.1% higher than the mean value, respectively). L2 and L11 showed the highest DW percentage in the adult stage (2.3% and 1.3% over the mean value, respectively). CL3 and CL5 in the baby and adult stage, respectively, had the lowest percentage of dry biomass (nearly 2.0% lower than their mean values).



*** Fig. 2.** Dry weight (DW) in the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ by the LSD test.

3.2. Nutraceutical compounds and antioxidant capacity

3.2.1 Total chlorophyll concentration

The highest chlorophyll content (Table 2) was recorded in the baby stage (mean value 27.7% and 15.8% higher than microgreens and adults, respectively) in all the varieties but CL2 (Fig. 3A), with no significant differences with the adult stage in some varieties (CL3, L1, L2, L3).

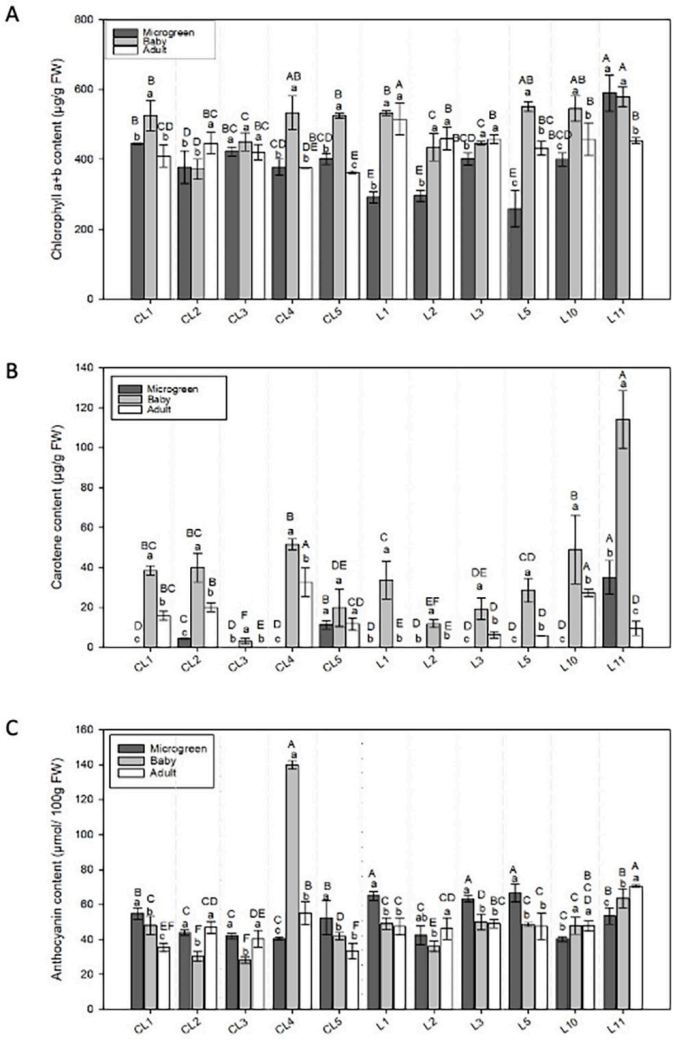
In the microgreen stage, landrace L11 had the highest Chl content (49.9% over the mean), while the lowest values went to L1, L2 and L5 (282.4±19.9 μg g^{-1} FW; 23.9% under the mean value) (Fig. 3A).

The highest Chl levels in the baby plants were obtained in CL4, L5, L10 and L11, while CL2 had the lowest values. Adult lettuces L1 and CL5 were highlighted for their highest and lowest Chl contents, respectively.

	Unit/Scale		Mean		Range	CV (%)	F-Ratio
Microgreen							
DW	%	7.53	±	0.59	5.81-8.84***	7.84	4.81
Chl	µg g^{-1} FW	393.77	±	92.38	200.89-647.87***	23.46	29.45
Car	µg g^{-1} FW	3.70	±	9.62	0-43.48***	259.627	70.1
Ant	µmol 100g^{-1} FW	50.67	±	10.29	36.12-72.34***	20.31	20.55
AsA	mg 100g^{-1} FW	61.05	±	11.61	34.26-87.01***	19.02	54.38
Phe	mg g^{-1} DW	18.69	±	5.23	7.43-28.78***	28.01	24.3
DPPH	%	71.52	±	21.17	12.65-88.46***	29.61	32.08
Ca	mg g^{-1} DW	9.76	±	1.75	6.61-12.81***	17.96	22.48
K	mg g^{-1} DW	48.23	±	3.93	39.54-60.08**	8.15	2.97
Fe	µg g^{-1} DW	225.08	±	60.74	68.1-362.18***	26.99	132.5
Baby							
DW	%	10.14	±	1.14	7.65-12.74***	11.26	13.03
Chl	µg g^{-1} FW	502.85	±	65.60	339.05-606.61***	13.05	14.63
Car	µg g^{-1} FW	36.76	±	28.89	1.87-126.98***	78.6	36.93
Ant	µmol 100g^{-1} FW	56.16	±	29.88	26.46-141.96***	56.19	296.27
AsA	mg 100g^{-1} FW	58.35	±	11.19	39.25-86.49***	19.18	34.83
Phe	mg g^{-1} DW	18.43	±	8.49	5.58-36.11***	46.06	45.24
DPPH	%	71.07	±	13.46	32.61-87.02***	18.93	23.79
Ca	mg g^{-1} DW	7.23	±	1.28	5.39-10.07***	17.65	7.39
K	mg g^{-1} DW	28.18	±	3.60	22.21-36.82*	12.78	2.53
Fe	µg g^{-1} DW	104.64	±	18.07	71.24-146.55***	17.27	4.28
Adult							
DW	%	6.48	±	1.46	3.83-13.25***	22.5	6.84
Chl	µg g^{-1} FW	434.36	±	46.61	359.85-564.58***	10.73	7.88
Car	µg g^{-1} FW	11.11	±	11.01	0-39.73***	99.1	35.03
Ant	µmol 100g^{-1} FW	47.63	±	10.50	29.15-71.76***	22.08	12.65
AsA	mg 100g^{-1} FW	34.45	±	14.33	13.44-64.2***	41.58	88.68
Phe	mg g^{-1} DW	3.91	±	2.50	0.56-9.38***	63.89	49.97
DPPH	%	15.88	±	9.55	3.58-39.41***	60.14	19.21
Ca	mg g^{-1} DW	8.87	±	1.79	6.13-13.79***	20.18	23.04
K	mg g^{-1} DW	59.89	±	7.66	44.36-75.96***	12.8	11.55
Fe	µg g^{-1} DW	171.44	±	114.32	74.51-514.25***	66.68	131.98

Antioxidant capacity; Ca: Calcium; K: Potassium; Fe: Iron.

*** Table. 2.** Variation parameters for the quality traits in the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Statistics were performed per stage. Values represent the mean, range, coefficient of variation (CV, %), F-ratio and significance (***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$) for the quality traits. DW: Dry weight; Chl: Chlorophylls; Car: Carotenes; Ant: Anthocyanins; AsA: Ascorbic Acid; Phe: Phenols; DPPH:



*** Figure 3.** The (A) chlorophyll a + b (Chl), (B) carotenoid (Car) and (C) anthocyanin (Ant) concentrations in the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ by the LSD test. FW: Fresh weight.

3.2.2 Carotenoids

Table 2 shows the Car content in the different development stages, which was higher in the baby stage than in the other development formats (mean values of 893.5% and 230.9% higher than microgreens and adults, respectively).
In the microgreen stage (Fig. 3B), three of the 11 varieties (CL2, CL5, L11) contained Car compounds, which were not detectable in the other varieties. In the baby stage, all the plants contained Car, which were remarkable in L11 (114.5 μg g^{-1} FW, 210.5% over the mean value), and also in L10 and three commercial varieties (CL1, CL2, CL4) for ranging between 38.5 and 48.9 μg g^{-1} FW. Of the adult lettuces, CL4 and L10 showed the highest Car level, which was not detectable in three varieties: CL3, L1 and L2.

3.2.3 Anthocyanins

One detected trend was the highest Ant content in the commercial varieties and landraces in the microgreen stage (mean values of 50.7 μmol $100g^{-1}$ FW and 56.2 μmol $100g^{-1}$ FW, respectively, Table 2), expect in CL4, L10 and L11, which was higher in the baby and adult stages (Fig. 3C).

Of the microgreens, three local landraces (L1, L3, L5) stood out for their high Ant level (64.9±1.7 μmol $100g^{-1}$ FW, Fig. 3C). In the baby stage, it was notably elevated in CL4 (148.9% higher than the mean value) and low in CL2 and CL3 (nearly 45% lower than the mean). Of all the adult plants, L11 had the highest Ant content (48.33% over the mean) and CL5 contained the least (29.94% under the mean).

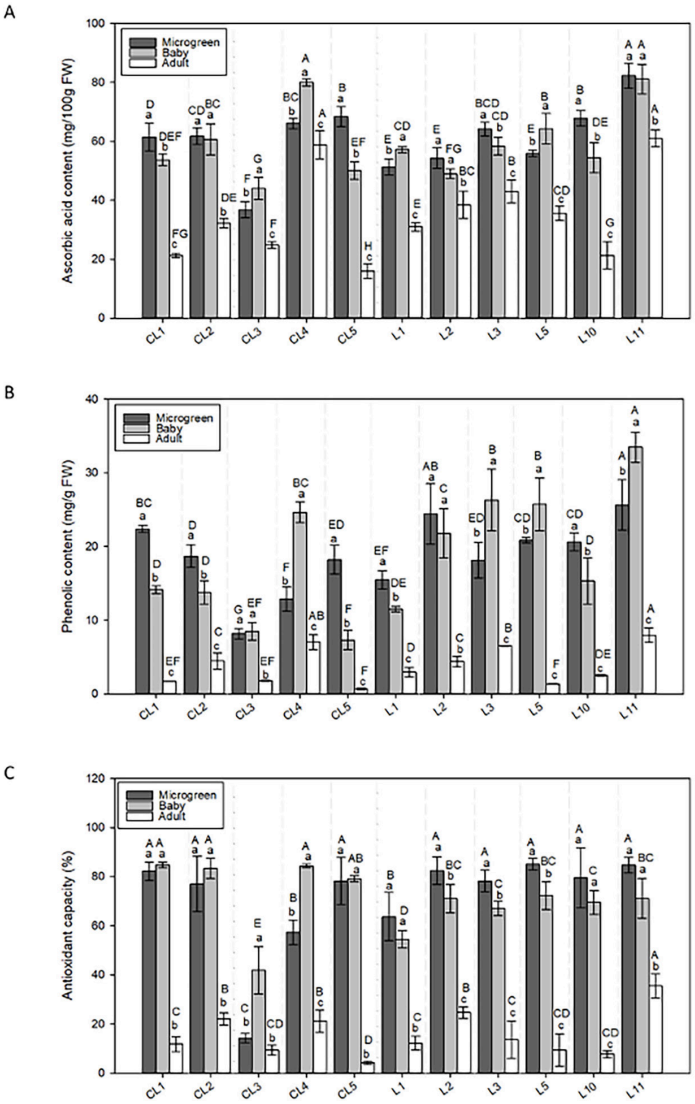
3.2.4 Ascorbic acid

The maximum AsA concentration appeared in the microgreen and baby stages (Table 2), and L11 presented the highest AsA levels (34.8% and 39.1% higher than the mean value for both stages, respectively) (Fig.4A). CL4 also had a high AsA level in the baby stage. The AsA concentration in adult lettuce (mean value 34.45 mg $100g^{-1}$ FW, Table 2) dropped in both the commercial and local landraces, with the lowest values for CL5 (53.8% lower than the mean). The highest AsA content in the adult stage was observed in lettuces CL4 and L11 (70.7% and 77.0% higher than the mean value, respectively).

3.2.5 Phenols

The mean Phe content (Table 2,) was similar in the microgreen and baby stages, and was around 79% higher than in adult lettuces. Two landraces (L2 and L11) stood out for their high Phe content in the microgreen stage (around 25.0 mg g^{-1} FW) (Fig 4B). In the baby stage, the most remarkable varieties were CL4 and local landraces L3 and L5, especially L11 (between 24.7 and 33.5 mg g^{-1} FW). CL3 had the lowest Phe content in both the microgreen and baby stages (56.3% and 53.9% lower than the mean, respectively). The highest Phe content in the adult stage was observed in lettuces CL4 and L11 (79.8% and 104.1% higher than the mean value, respectively). Three of the five commercial varieties (CL1, CL3, CL5) obtained very low Phe contents in the adult stage (from 0.7 to 1.8 mg g^{-1}FW), which occurred only in one local landrace:

L5 with 1.4 mg g-¹FW.



*** Figure 4.** The (A) ascorbic acid (AsA), (B) phenols (Phe) and (C) antioxidant (DPPH) capacity in the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ by the LSD test. FW: Fresh weight.

3.2.6 Antioxidant capacity

Like AsA and Phe contents, the greatest DPPH activities in the commercial varieties and landraces appeared in the microgreen and baby development stages (mean values of 71.5% and 71.1%, respectively, Table 2), expect in CL3, which was higher only in the baby stage. No significant differences between the microgreen and adult stages were found (Fig. 4C). Lower antioxidant capacity was measured in the adult stage (around 55.6% lower than in the other two stages, Table 2). When comparing varieties in their different development formats, the top DPPH levels in the microgreen lettuces were for eight of the 11 varieties, except CL3, CL4 and L1 (Fig. 4C). In this stage, most local landraces presented between 6.8% and 13.6% more DPPH activity than the mean (71.5%) and was slightly lower in only L1 (7.8%).

For the baby stage, the lowest DPPH was displayed in CL3 and L1 (29.1% and 16.5% lower than the mean, respectively). In the adult stage (mean value 15.9%, Table 2), the greatest activity was observed in L11 (19.7% higher than the mean value) with a significant difference ($p<0.05$).

3.3. Mineral concentration

Table 3 shows the concentration of three of the main minerals (Ca, K, Fe) related to lettuce nutritional quality. The results for the other macro- and micronutrients appear in Supplementary Table 1.

The maximum Ca concentration was found in the microgreen stage in all the varieties (Table 2) but L11, which was exceeded by adult lettuce. Of the varieties, four of the six local landraces (L2, L5, L10, L11) and one commercial lettuce (CL3) in the microgreen stage obtained the highest Ca concentrations (between 10.28 and 12.38 mg g^{-1} DW, Table 3), while the lowest value was for L1 (26.42% lower than mean value, Table 2). In the baby stage, the most remarkable varieties were CL3, CL4, L4, L5, L10 and L11 (between 7.76 and 8.89 mg g^{-1} DW). The Ca content in adults was also high in CL4 and two other local landraces (L1 and L3).

As a general trend, the adult stage presented the highest K concentration, followed by microgreens, with the lowest levels in the baby stage (52.15% and 41.57% lower than the previous ones, respectively) (Table 2). The highest K concentrations in microgreens were obtained in CL3, CL5, L3, L5 and L10 (between 48.9 and 53.6 mg g^{-1} DW, Table 3). The top K levels in the baby stage were for varieties CL3, CL4 and L2 (between 30.3 and 32.5 mg g^{-1} DW). In the adult stage, two commercial varieties (CL2 and CL3) and two local landraces (L1 and L3) stood out for their high K content (between 66.0 and 68.7 mg g^{-1} DW).

Another trend was the lowest Fe concentrations, which were recorded in the baby stage (mean value 53.5% and 39.0% lower than the microgreen and adult stages, respectively, Table 2). Depending on variety, the highest significant Fe levels (Table 3) were obtained in the microgreen stage (CL1, CL2, CL5, L2 and L3), the adult stage (CL3, CL4 and L11), or both (L1, L5 and L10). Of the microgreens, varieties L2 and CL3 stood out for presenting the highest and lowest Fe concentrations (344.9 and 77.7 μg g^{-1} DW, respectively, Table 3). In the baby stage, the Fe concentration was more homogeneous (low CV%, Table 2) and five of the 11 varieties (CL1, CL3, CL4, L2 and L11) had top levels (between 114.7 and 122.5 μg g^{-1} DW). The highest Fe concentration in adult lettuces was for local landrace L11 (187.8% higher than the mean adult value, Table

2). Three commercial varieties and one local variety (CL1, CL2, CL5 and L3) presented the lowest Fe level in the adult stage (between 50.8% and 35.8% lower than mean).

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Variety	State	Ca (mg g^{-1} DW)				K (mg g^{-1} DW)				Fe (µg g^{-1} DW)			
CL1	Microgreen	8.76 ± 0.87	DEF	a		48.06 ± 1.26	B	b		230.48 ± 20.05	CD	a	
	Baby	5.64 ± 0.16	C	c		26.47 ± 3.03	CD	c		119.34 ± 16.77	A	b	
	Adult	7.21 ± 0.03	E	b		58.13 ± 2.63	C	a		108.80 ± 7.19	DE	b	
CL2	Microgreen	8.66 ± 0.54	DEF	a		45.85 ± 4.56	BC	b		230.51 ± 2.06	CD	a	
	Baby	6.65 ± 0.82	BC	b		25.37 ± 3.35	D	c		92.90 ± 9.12	C	b	
	Adult	7.19 ± 0.17	E	b		68.73 ± 4.84	A	a		84.30 ± 10.40	E	b	
CL3	Microgreen	10.91 ± 1.35	B	a		53.59 ± 4.36	A	b		77.74 ± 10.88	G	b	
	Baby	7.98 ± 0.82	A	b		32.53 ± 2.61	A	c		114.69 ± 8.51	AB	b	
	Adult	8.76 ± 0.65	CD	b		66.02 ± 4.46	AB	a		218.88 ± 38.54	B	a	
CL4	Microgreen	9.66 ± 1.29	C	a		47.14 ± 2.89	B	b		155.56 ± 4.73	F	b	
	Baby	7.76 ± 0.47	A	b		31.86 ± 1.87	AB	c		118.73 ± 6.45	A	b	
	Adult	11.07 ± 0.70	B	a		57.91 ± 6.47	C	a		193.38 ± 12.79	B	a	
CL5	Microgreen	8.21 ± 0.23	EF	a		48.92 ± 3.79	AB	a		255.54 ± 12.62	B	a	
	Baby	5.88 ± 0.46	C	c		26.46 ± 2.02	CD	b		94.77 ± 16.44	C	b	
	Adult	6.82 ± 0.61	E	b		46.21 ± 1.68	D	a		110.06 ± 22.83	DE	b	
L1	Microgreen	7.72 ± 0.58	F	a		48.73 ± 4.69	B	b		216.87 ± 8.87	DE	a	
	Baby	6.23 ± 0.55	C	b		27.89 ± 1.65	BCD	c		91.43 ± 13.50	C	b	
	Adult	7.51 ± 0.83	E	a		66.53 ± 5.29	AB	a		123.30 ± 10.28	CD	ab	
L2	Microgreen	12.37 ± 0.15	A	a		47.69 ± 0.86	B	a		344.91 ± 13.64	A	a	
	Baby	8.89 ± 1.02	A	b		30.25 ± 5.41	ABC	b		119.75 ± 20.78	A	b	
	Adult	9.47 ± 0.71	CD	b		50.63 ± 1.14	D	a		119.34 ± 3.73	D	b	
L3	Microgreen	7.77 ± 0.79	EF	ab		49.18 ± 3.43	AB	b		244.98 ± 3.66	BCD	a	
	Baby	6.50 ± 1.03	C	b		28.21 ± 5.60	ABCD	c		98.21 ± 5.05	BC	b	
	Adult	8.78 ± 0.73	CD	a		66.10 ± 4.57	AB	a		100.87 ± 13.03	DE	b	
L5	Microgreen	12.38 ± 0.23	A	a		49.73 ± 2.88	AB	b		222.59 ± 9.10	DE	a	
	Baby	8.58 ± 1.18	A	b		25.48 ± 0.46	D	c		89.74 ± 13.70	C	b	
	Adult	9.70 ± 1.01	C	b		59.69 ± 4.42	C	a		153.94 ± 27.85	C	ab	
L10	Microgreen	10.66 ± 0.15	BC	a		49.57 ± 1.33	AB	b		208.97 ± 2.41	E	ab	
	Baby	7.77 ± 0.68	AB	b		26.68 ± 1.41	CD	c		88.93 ± 12.13	C	b	
	Adult	8.65 ± 0.92	D	b		56.93 ± 4.46	C	a		152.15 ± 19.59	C	a	
L11	Microgreen	10.28 ± 0.12	BC	b		42.13 ± 2.81	C	b		236.34 ± 9.73	BCD	b	
	Baby	8.07 ± 1.09	A	c		28.77 ± 2.17	ABCD	c		122.49 ± 17.73	A	b	
	Adult	12.41 ± 0.92	A	a		61.91 ± 1.62	BC	a		493.47 ± 23.01	A	a	

*** Table 3.** The calcium (Ca), potassium (K) and iron (Fe) concentrations in the collection of the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Values are the mean±SE of four replicates per variety. The means are subjected to a one-way ANOVA analysis. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ using the LSD test. DW: Dry weight.

3.4. PCA Analysis

The PCA and the eigenvalues higher than 1 reflected a different pattern in the correlation of lettuces in the three development stages (Table 4). In all cases, there were three significant PCs that described around 83%, 75% and 76% of the variability between varieties for the microgreen, baby and adult stage, respectively

Microgreen	First PC	Second PC	Third PC
DW	0.355	-0.230	
Chl a+b	0.203	**-0.557**	
Car	0.334	**-0.294**	0.213
Ant			**-0.665**
AsA	**0.419**		
Phe	0.366	0.352	
DPPH	0.373	0.346	
Ca		0.270	**0.653**
K	**-0.409**		-0.182
Fe	0.295	**0.435**	
Eigenvalue	4.70	2.28	1.35
Variance explained (%)	46.99	22.80	13.49
Cumulative variance explained (%)	46.99	69.79	83.28

Baby	First PC	Second PC	Third PC
DW	0.201	**-0.471**	
Chl a+b	0.292		**0.509**
Car	**0.459**		0.221
Ant	0.335		**-0.545**
AsA	**0.510**		
Phe	**0.413**	0.167	0.180
DPPH	0.284	-0.269	**-0.466**
Ca		**0.403**	0.292
K		**0.553**	-0.153
Fe	0.169	**0.436**	-0.179
Eigenvalue	3.46	2.64	1.39
Variance explained (%)	34.60	26.43	13.90
Cumulative variance explained (%)	34.60	61.02	74.93

Adult	First PC	Second PC	Third PC
DW	0.328		-0.326
Chl a+b	**-0.360**		
Car		**0.593**	**-0.415**
Ant	0.331	-0.270	0.242
AsA	**0.396**	0.157	
Phe	**0.369**	0.334	
DPPH	**0.406**		
Ca	0.248	**-0.428**	-0.218
K		0.388	**0.718**
Fe	0.313	-0.289	0.283
Eigenvalue	5.09	1.44	1.07
Variance explained (%)	50.88	14.40	10.73
Cumulative variance explained (%)	50.88	65.27	76.00

*** Table 4.** Correlation coefficients for the quality traits of the three first principal components, eigenvalue, and the relative and cumulative proportions of the total variance explained by these components, in the collection of the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). DW: Dry weight; Chl: Chlorophylls; Car: Carotenoids; Ant: Anthocyanins; AsA: Ascorbic Acid; Phe: Phenols; DPPH: Antioxidant capacity; Ca: Calcium; K: Potassium; Fe: Iron.

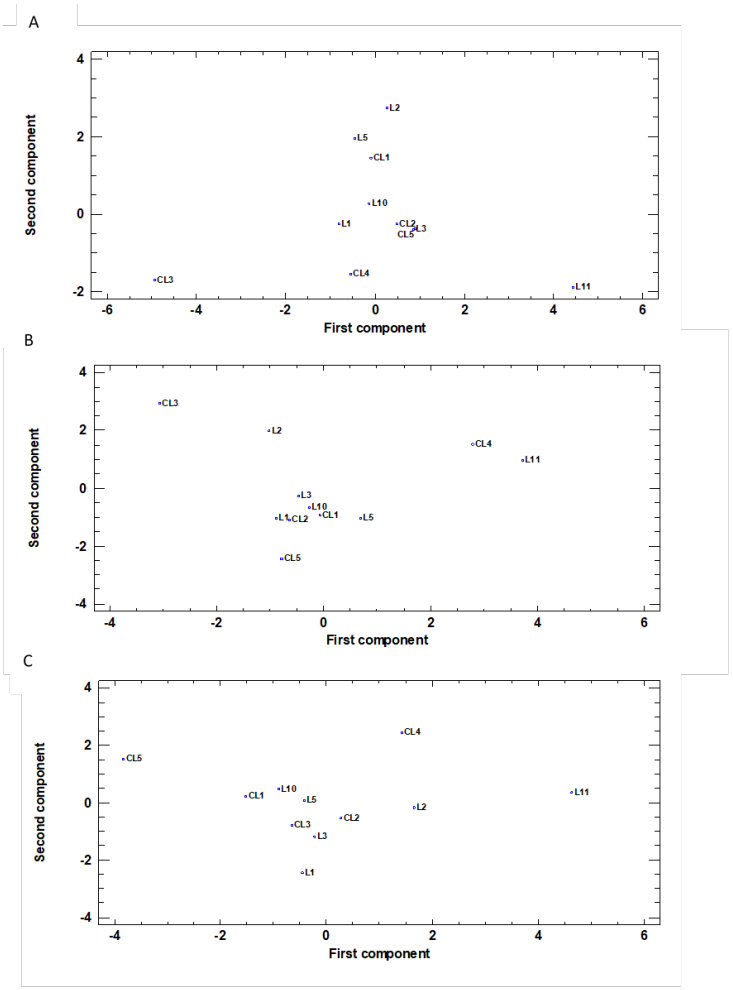
In microgreens, the first, second and third PCs accounted for 46.9%, 22.8% and 13.5% of the total variation for the studied traits, respectively. The first PC correlated positively with all the traits, and the AsA concentration had the highest value (0.419), but negatively with the K concentration (-0.409). When analysing the second PC, the highest positive correlation was recorded for the Fe mineral (0.435), with negative correlations for Chl and Car (-0.557 and -0.294, respectively).

In the baby stage, the relevance of the first PC was lesser than in the other two stages and accounted for only 34.6% of total variation. The second and third PCs accounted for 26.4% and 13.9% of variability, respectively. Regarding the correlation values in the first PC, all the traits were positively correlated, and the most significant results were obtained for the Car, Phe and AsA concentrations. The highest positive correlations in the second PC corresponded to mineral contents (Ca, K and Fe), while DW was negatively correlated (-0.471).

The distribution of the adult lettuces in the PCA was located mostly by the variability of the traits in the first PC (50.9%), while the second and third PCs represented only 14.4% and 10.7% of variation, respectively. Most traits presented a moderate positive correlation of the first PC, and DPPH, Phe and AsA had the highest values (between 0.406 and 0.369). A moderate value was also obtained for the negative correlation (-0.360) with Chl content of the first PC. When analyzing the second PC, the highest positive correlation was for Car concentration (0.593), while the most negative value went to

Ca content (-0.428).

For three development stages, the projection on the PCA plot for the first and second PCs (Fig. 5) showed a similar pattern of spread over the area. In general terms, there was a group with a large number of varieties located in the central zone of the graphs, while two or three varieties were located further to the right or the left of the plots.



*** Fig. 5.** The principal component analysis (PCA) for the 11 lettuce varieties based on the quality traits represented in the two first components (first component, X-axis; second component, Y-axis) of the PCA for the A) microgreen stage (46.99% and 22.80% of total variation, respectively), B) baby stage (34.60% and 26.43% of total variation, respectively) and adult stage (50.88% and 14.44% of total variation, respectively).

In microgreens, the highest value for the first PC (right zone) was recorded for landrace L11 (Fig. 5A) and correlated with its top levels for four traits: Car, AsA, Phe and DPPH (Figs. 3B and 4A-C) and low K levels (Table 3). On the contrary, the lowest values for AsA, DPPH and Phe throughout the experiment located variety CL3 further to the left of the plot. For the second PC, local landrace L2 was at the top of the plot for its high Fe content (Table 3) and low Chl concentration (Fig. 3A), while L11 was at the bottom for its high pigments concentration (Chl and Car, Figs. 3A,B). The low Fe level in CL3 and CL4 (Table 3) also placed these two commercial varieties in the lower graph area (Fig. 5A).

In the baby stage, L11 was located on the right (Fig. 5A) for its high levels of Car (Fig. 3B), AsA and Phe (Fig. 4A,B). CL4 also presented good Car and AsA levels. The lowest Car, AsA and Phe concentrations in the experiment of lettuce CL3 placed this commercial variety further left in the plot. For the second PC, these three varieties together with L2 were placed at the top of the plot for having good concentrations of minerals (Ca, K and Fe, Table 3), and for CL3 also presenting the lowest dry biomass percentage in the baby stage (Fig. 2). In contrast, the high DW value of CL5, together with a low mineral content, left this commercial variety at the bottom of the plot.

L11 in the adult stage once again stood out for presenting the highest AsA, Phe and DPPH levels, and it was located further right in Fig. 5C, followed by CL4 and L2 with good levels for these traits. Unlike the other two stages, the variety further to the right in the adult format was CL5 for presenting the lowest AsA content throughout the experiment, together with low Phe and DPPH levels (Fig. 4A,B,C). According to the second PC, the most remarkable variety was CL4 (top of the plot) for occupying the first and second places for Phe and Car contents. L1 (bottom of the plot) was also outstanding for having one of the lowest Car concentrations during the experiment in the adult stage.

3.5. Correlation between quality compounds

Correlation analyses were carried out to estimate the relation between the most important quality traits in the three development stages (Table 5).

In microgreens, the pairwise coefficients showed a positive correlation and a statistical significance for six pairs of traits of the 36 studied ones. The most representative positive relations were observed between Phe and DPPH (r= 0.721) and between Car and AsA (r= 0.505). Statistically significant negative correlations for pairs of traits were also determined in four of the 45 studied ones. The closest negative relations were for K vs. Fe concentrations (r= -0.604) and K vs. Car (r= -0.494).

In the baby stage, the number of positive correlations rose to 13 and the strongest coefficients were observed in AsA vs. Car, AsA vs. Ant and Asa vs. Phe (r between 0.615 and 0.697).

All the significant pairwise coefficients in the adult lettuces showed positive correlations and the number came to 17 pairs of traits of the 36 studied ones. The most representative relations were observed between the several Ant, AsA, Phe and DPPH combinations, with the highest values in the pairs AsA vs. Phe, AsA vs. DPPH and Phe vs. DPPH (r between 0.867 and 0.703). Important relations (r between 0.519 and 0.799) appeared between minerals (Ca and Fe) and for several quality traits (Ant, AsA, Phe and DPPH).

Microgreen

	Chl a+b	Car	Ant	AsA	Phe	DPPH	Ca	K	Fe
Chl a+b		**0.4143***	-0.0364	**0.3928***	0.0747	0.0346	-0.117	-0.119	-0.0901
Car			0.0732	**0.5045****	0.1708	0.2554	-0.1941	**-0.4941****	0.2132
Ant				0.0124	0.2169	0.1641	**-0.4458****	-0.1202	0.2338
AsA					**0.3244***	**0.4498****	-0.1398	**-0.3722***	0.1853
Phe						**0.7212*****	0.262	-0.2496	0.2812
DPPH							0.1729	-0.1802	0.1598
Ca								0.0285	0.0943
K									**-0.604*****
Fe									

Baby

	Chl a+b	Car	Ant	AsA	Phe	DPPH	Ca	K	Fe
Chl a+b		**0.5116****	0.2391	**0.3653***	0.27	0.0557	0.0786	0.0099	0.0448
Car			0.271	**0.6154*****	**0.4764****	0.2258	0.1565	-0.0187	**0.4546****
Ant				**0.6243*****	0.2022	**0.4963*****	-0.0116	0.1843	0.116
AsA					**0.6974*****	**0.4557****	0.1692	-0.0295	**0.3639***
Phe						0.184	**0.3394***	0.0149	**0.3192***
DPPH							-0.1262	-0.2486	0.0874
Ca								**0.4761****	0.1332
K									0.2664
Fe									

Adult

	Chl a+b	Car	Ant	AsA	Phe	DPPH	Ca	K	Fe
Chl a+b		-0.3158	**0.3714***	0.2159	0.1143	0.2218	0.0939	**0.419****	-0.0149
Car			0.15	0.0572	0.2014	0.0254	0.147	-0.0818	0.0858
Ant				**0.5291*****	**0.4224****	**0.5695*****	**0.5614*****	0.2667	**0.584*****
AsA					**0.8672*****	**0.7029*****	**0.7997*****	0.2131	**0.5939*****
Phe						**0.7243*****	**0.6467*****	0.1911	**0.5194*****
DPPH							**0.5732*****	0.0936	**0.5226*****
Ca								0.0815	**0.7591*****
K									0.0814
Fe									

*** Table 5.** Linear correlation coefficient (r) and its significance between the quality traits in the collection of the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). ***, **, * indicate significance at p<0.001, p<0.01, p<0.05 for r. Chl: Chlorophylls; Car: Carotenoids; Ant: Anthocyanins; AsA: Ascorbic Acid; Phe: Phenols; DPPH: Antioxidant capacity; Ca: Calcium; K: Potassium; Fe: Iron.

4. DISCUSSION

Although lettuce is particularly known for its high water percentage and low calorie content [5], as is generally consumed and marketed whole and raw [26], more nutrients are preserved than in other cooked or processed vegetables. Thus its nutritional benefits related to its dietary fiber, mineral and vitamin contents, plus several bioactive compounds like carotenoids and phenolic compounds, remain [5].

As several authors like Mou [27], Kim et al. [5], Kiriacou et al. [28,29], Wojdylo et al. [30] mention, nutrient content of lettuce is determined by genetics, environmental influence, genotype-environment interactions and plants' harvest stage. Microgreens and baby lettuces may have much higher levels of vitamins, minerals and other health beneficial phytonutrients than mature leaves. For these reasons, these types of seedlings are now appreciated as functional foods [31–35]. Seeds are a source of proteins, carbohydrates and sometimes fats, but not vitamins [36]. However, germination and embryo growth promote intense metabolic activity in seed, in which several chemical reactions take place, including enzyme synthesis. Most carbohydrates and fats are reused in the synthesis of vitamins, sugars, proteins and mineral salts [36]. It is because of these processes that seedlings are considered functional foods with substantial health-promoting properties [33]. This statement is reflected in our study because varieties' antioxidant capacity, including the main antioxidant compounds like ascorbic acid and phenols, follows a clear pattern that is repeated in all cultivars insofar as microgreens and baby greens present higher antioxidant properties than adult plants.

Phenols and ascorbic acid serve as scavengers of reactive oxygen species for protecting young expanding leaves that are prone to light damage [17]. Phenolic compounds also seem to influence the sensory qualities of microgreens. In this regard, Xiao et al. [37] reported that the total Phe concentration correlates with overall eating quality and several aspects of sensory qualities. Leafy vegetable microgreens present from 2- to 5-fold more nutrients than mature leaves from adult vegetables, according to Manjula et al. [38]. In our study, this tendency is proven because Phe content was almost 5-fold higher in seedlings than in adult lettuces regardless of the variability observed among varieties. Our results about adult lettuces fall in line with Liu et al.[39] , Mmapholo et al. [26], Huang et al. [40] and Kim et al. [5], who claim that red-leaf adult cultivars are richer in total Phe content. This event is repeated in our trial as highlighted by varieties CL4 and L11. These results are higher than the values reported by several authors for red lettuce cultivars [5,26,27,41] and are also higher than the values obtained from vegetables and fruit known for their elevated Phe content, such as spinach (2.69 mg g-1 FW) [42], red onion (2.53–3.11 mg g-1 FW) [43], strawberry (3.64 mg g-1 FW) [43], plum (3.04 mg g-1 FW) and blueberry (4.25 mg g-1 FW) [44]. In the initial development stages, the Phe content of varieties CL4 and L11 is similar to that of other varieties a priori qualified as less reddish, which is the case of L3 and L5 in the baby green stage. However compared to the Phe content in many other cultivars, microgreens like beetroot (166 mg g-1 FW) or amaranth (586 mg g-1 FW) [30], lettuce microgreen cultivation is not remarkable. This fact does not seem to affect consumer choice because lettuce and carrot appear among the most preferred microgreens, followed by green peas, red amaranth and finger millet [45].

Likewise, significant differences were detected when determining the total vitamin C concentration of varieties. In addition, AsA content was 41% higher in the seedling stages than in adult lettuces. Like phenols, red varieties CL4 and

L11 stood out from the rest. Similarly, when comparing our results to those of other authors [27,46,47], the obtained values, especially those in landraces, for vitamin C content were higher when comparing our varieties to equivalent ones in terms of lettuce type, and based mainly on colour or head structure, in other articles. These results come close to the vitamin C values obtained in other species: peas (30.9 mg 100g-1 FW), spinach (31.6 mg 100g-1 FW), green beans (15.1 mg 100g-1 FW) [48]; grapefruit (39.0 mg 100g-1 FW), banana (11.1 mg 100g-1 FW), mango (37.0 mg 100g-1 FW) [49]. Even the coloured lettuce varieties obtain the vitamin values of those crops known for their high ascorbic content. For example: orange (49.4 mg 100g-1 FW); pepper (50.3 mg 100g-1 FW) [50]; mandarin (57.4 mg 100g-1 FW); blueberry (60.1 mg 100g-1 FW) [44]. Along the same lines, when comparing the values obtained in the microgreens and baby greens, even the values obtained in microgreens and baby greens equalled those obtained for broccoli (77.1 mg 100g-1 FW) [48] and strawberry (77.3 mg 100g-1 FW) [49]. These values are also comparable to those detected in other microgreens species: carrot (65.6 mg 100g-1 FW); onion (29.9 mg 100g-1 FW); spinach (71.2 mg 100g-1 FW); radish (88.5 mg 100g-1 FW) [38].

Antioxidant capacity followed the same pattern as the phenolic compounds and vitamin C contents, and was more prominent in the microgreen and baby green stages. However, as no quantification was carried out, we were unable to make a comparison to other crops, and only a comparison of the varieties under study was feasible. As previously mentioned, landrace L11 stood out in the adult stage. These data imply that both phenols and vitamin C can be determinants for the generally increased antioxidant capacity of this crop because the varieties that stood out for these nutraceutical compounds tended to have a much higher antioxidant capacity.

Similarly, some of the analysed pigments are also apparently involved in total antioxidant capacity, especially anthocyanins, which are actually the phenolic compounds that abound in red-coloured lettuce [51–53]. Moreover, several authors like Llorach et al. [41], Baslam et al. [54], and Kim et al. [5] claim that red pigmentation is indicative of total Ant and Phe content, which also corroborates the correlations found between these two parameters, together with the total antioxidant capacity in the baby and adult lettuces. Nevertheless, no correlations were found in microgreens, perhaps because at the time of seedlings' initial growth, metabolic activity intensifies after germination [36], and anthocyanins begin to be synthesised, together with the other phenolic compounds, but their antioxidant properties are still irrelevant. In addition, a strong genetic component, or environmental factors like light, may also affect the synthesis and activity of anthocyanins because the anthocyanins concentration does not seem to follow a clear pattern, but varies among varieties in different ways.

The nutritional value of lettuce varies for different varieties and environmental conditions [5,29,36,55]. Of environmental factors, light is one of the most important variables to affect phytochemical concentrations in plants. Light conditions influence the morpho-physiology of microgreens, together with the biosynthesis and accumulation of phytochemicals [56–58]. According to Mou and Ryder [10], the lower nutritional value of some varieties is due to the marked enclosure of their leaves in the head structure as most of the edible head structure portion includes leaves that are not exposed to light. Moreover, the size and number of external leaves, as well as head type, lead to differences in the light microenvironment between outer and inner leaves [54]. One clear example is the lower nutrient content of crisphead lettuce versus romaine types [59]. Of the varieties included in our study, only CL5, a commercial iceberg variety, was confirmed as having the lowest values of vitamin C, DPPH, Chl, DW and Phe. It was undoubtedly

the variety with the highest degree of leaf overlap in our study. However, variety CL4, a variety with the most patent buds, stood out for its high proportion of nutraceutical compounds. This could be due to its characteristic purple colour, which would be indicative of high Ant and Car contents. Conversely, Roman purple variety L11, which was the variety with the lowest degree of leaf overlap, stood out in almost every analysed phytochemical. Indeed the different degrees of leaf overlap between our varieties could have influenced the variability of the studied compounds in the adult stage. Likewise, as our analyses were carried out in different lettuce development stages, the nutritional quality pattern between varieties was not maintained as no head structure was present in the youngest stages (microgreens and baby). This meant that varieties were highlighted when microgreens were not necessarily the most outstanding when in the baby leaf or adult stages. In this regard, as consumers, food nutritionists and producers are showing more interest in the health-related effects of the products they eat [6], the information herein presented could be helpful to guide consumers in their diet choices.

As mentioned earlier, the anthocyanin synthesis rate appeared to be variety-dependent as no firm pattern appeared for production throughout development for the studied varieties. For microgreens, narrow variability was observed between varieties and Ant content did not seem proportional to the colour of these seedlings, which occurred in more advanced development stages. This implies that other pigments absorbed at the same wavelength as anthocyanins and were synthesised on a large scale. This could interfere with colour determination in microgreens. Some perfect examples of this statement lie in varieties CL4 (with a completely red first true leaf) and L1 (completely green coloured seedlings). Landrace L1 had the highest Ant concentration in our study (65 μmol $100g^{-1}$ FW), while CL4 matched the varieties with the lowest concentration (36.8 μmol $100g^{-1}$ FW) in the microgreen stage. In the following development stages, the reddish plant colouration was in accordance with the anthocyanin measured concentration. Variety CL4 in the baby green stage (155 μmol $100g^{-1}$ FW), and landrace L11 in the adult stage (70.65 μmol $100g^{-1}$ FW) are highlighted and, thus, confirm the theory that red lettuce colouration is indicative of Ant content [5,41,54,60].

This also supports the notion that the higher Ant content, the more light exposure [60], which was favoured by low degrees of leaf overlap (open lettuce vs. crisphead formats), in addition to longer exposure times (adult vs. early stages).

As previously reported, differences in the carotenoids content in lettuce types has been suggested to be related to head structure because it is regulated by light [5,10]. In addition, the increase in these pigments is beneficial due to their antioxidant properties [26,61]. In the juvenile development stages, the positive correlation between carotenoids and phenols was irrelevant, but the trend was positive and became statistically significant when plants reached maturity. This indicates the contribution of these pigments to total antioxidant activity. This event has also been observed in ginger [62] and palm oils [63]. In our study, no direct correlation between carotenes and the antioxidant capacity of lettuce in all the development stages could be due to the antioxidant role of carotenes not being as relevant as that of phenols, anthocyanins or vitamin C. Regardless of the observed wide inter-varietal variability, the carotenoids content in microgreens was practically null, while the highest values went for baby plants. According to Wojdylo et al. [30], these results are unexpected because he claims that microgreens contain high levels of carotenoids and chlorophylls, among others. This could indicate that the machinery used for carotenoid production is late-activated during development. Once again, the need to know each variety and its optimum harvesting period to obtain the best

nutritional benefit from them is highlighted. The highest Car values were for varieties L11 (114.2 µg g-1 FW), CL4 (51.5 µg g-1 FW) and L10 (48.9 µg g-1 FW) in the baby stage. In particular, the value obtained in landrace L11 was comparable even to the crops known for their high carotenoid contents, such as red peppers (63-130 µg g-1 FW) [64,65]and carrots (95.9 µg g-1 FW) [66].

As far as Chl content is concerned, it has been shown to depend not on light itself, but also on the quality of this resource [67]. Similarly, it has been demonstrated that Fe is responsible for the biosynthesis of this pigment, at least the water-soluble Fe fraction [68]. Fe-deficient plants are usually characterised by developing marked chlorosis, which lowers both chlorophyll and carotenoid concentrations [69]. However, in our study we obtained a negative relation between Chl content and the total Fe concentration, which became more pronounced in the adult plant stage. When focusing on this development stage, the relation between Ant and Chl was also negative, while that with Fe was positive. As we did not work in an Fe-deficient environment, and as all the varieties ranged within the optimal Fe concentration for this crop in the adult stage (0.41-2mg 100 g -1 FW [59]), what all this might indicate is that reddish varieties are those with more capacity to absorb or accumulate Fe, likely thorough more efficient Fe acquisition or transport systems. This does not imply that greener ones are in Fe-deficit and are not, therefore, capable of producing Chls. One clear example of this statement would be CL2. This variety has completely green leaves and is one of the accessions with the highest Chl content in our study, but was ranked last for Fe concentration. There could also be other factors that affect this relation, such as the contents of other pigments like carotenes, or other minerals also related to chlorophyll synthesis like Mg, which is a structural constituent of chlorophylls [70].

For Ca, a similar trend to that observed for Fe was noted because the really striking relations between this mineral and other phytonutrients were detected in the adult stage. The Ca concentration was generally higher in microgreens. This finding would coincide with Pinto et al. [9]. In our assay, once plants reached maturity, the concentration of this mineral appeared to be correlated with the content of the main antioxidant compounds. The benefits deriving from Ca application are well-known, especially in postharvest activities because it maintains cell turgor, tissue firmness, delays the catabolism of membrane lipids [71], and reduces fruit browning [72] by prolonging the storage life of fresh fruit [73]. Similarly, there is evidence that Ca promotes anthocyanin synthesis in vitro [74]. Although these facts may support the relation between Ca and the major antioxidants in lettuce, Ca can be obtained from the substrate, and plays an essential role in plant development and overall plant health. In lettuce leaf tissue, an increase in Ca enhances both photosynthetic capacity and chlorophyll synthesis [75,76], which implies more primary product from photosynthesis as glucose and fructose [76].

Finally, K is one of three major nutrients required for normal plant growth, and is involved in plant photosynthesis, w by substrate K availability [77,78]. Our study did not carry out a comparative study between different substrate types. This only confirms that some varietal genetic differences enable them to capture and/or retain a certain K concentration What we were able to verify was the tendency to accumulate this mineral in lettuce leaves throughout development, which has been explained by Kyriacou et al. [34], Pinto et al. [9] and el El-Nakhel et al. [17]. The K concentration in mature leaves was much higher than in the microgreens for almost all the varieties under study.

5. CONCLUSIONS

By taking into account lettuce´s fraction of functional compounds and its high consumption rate, it constitutes a very interesting source of nutrients (minerals and functional compounds). The results of the present study show that nutrient content depends on lettuce type, colour and development stage. Comparative nutrient data of several popularly consumed lettuce cultivars were provided, which could assist consumers to make food choices with higher nutritional value.

Of all the studied varieties, landrace L11 stands out from all the others, and in all the studied stages, practically in all the analysed parameters. This shows the extremely high potential of this traditional reddish variety, and its interest for consumers because of its attractive colour. However, all except commercial variety CL4, for the other studied cases it would be advisable to promote trade in stages other than the adult stage. Some examples of this are: CL5, considered the most deficient in phytonutrients upon maturity, but it stands out for its DPPH and AsA content in microgreens; landrace L5, a variety that is not particularly remarkable in any analysis, is highlighted for its total DPPH and Chl, Car and AsA contents, in the baby green stage. In turn, this underlines the idea of the marked existing, but barely exploited variability of traditional varieties.

Variety	State	Na (mg g^{-1} DW)			Mg (mg g^{-1} DW)			P (mg g^{-1} DW)			S (mg g^{-1} DW)
CL1	Microgreen	2,26 ± 0,23	C	a	2,98 ± 0,22	BC	a	12,42 ± 0,83	AB	a	3,50 ± 0,24
	Baby	1,07 ± 0,11	D	c	1,45 ± 0,06	DE	c	3,62 ± 0,29	BC	c	1,43 ± 0,07
	Adult	1,92 ± 0,06	F	b	2,58 ± 0,31	BCD	b	7,16 ± 0,85	BC	b	1,34 ± 0,10
CL2	Microgreen	1,88 ± 0,11	D	b	2,64 ± 0,10	D	a	12,20 ± 0,35	ABC	a	3,91 ± 0,12
	Baby	1,18 ± 0,16	CD	c	1,45 ± 0,20	DE	c	3,46 ± 0,22	BC	c	1,41 ± 0,07
	Adult	2,68 ± 0,11	ABCD	a	2,28 ± 0,15	DE	b	5,27 ± 0,49	E	b	1,36 ± 0,03
CL3	Microgreen	4,24 ± 0,20	A	a	2,27 ± 0,01	E	a	8,38 ± 0,35	F	a	2,97 ± 0,23
	Baby	1,35 ± 0,06	BC	c	1,46 ± 0,23	DE	b	4,70 ± 0,46	A	c	2,07 ± 0,47
	Adult	2,74 ± 0,31	ABC	b	2,10 ± 0,11	E	a	5,64 ± 0,53	DE	b	1,94 ± 0,11
CL4	Microgreen	2,56 ± 0,17	bc	a	2,79 ± 0,29	CD	a	9,52 ± 1,45	EF	a	3,15 ± 0,60
	Baby	1,44 ± 0,09	B	b	1,81 ± 0,12	B	b	4,71 ± 0,63	A	c	1,94 ± 0,15
	Adult	2,93 ± 0,32	AB	a	3,11 ± 0,44	A	a	6,34 ± 0,46	CD	b	2,01 ± 0,07
CL5	Microgreen	1,72 ± 0,04	D	b	2,61 ± 0,06	D	a	10,73 ± 0,22	CDE	a	5,11 ± 0,10
	Baby	1,28 ± 0,26	BC	c	1,37 ± 0,18	E	c	3,00 ± 0,59	C	c	1,59 ± 0,08
	Adult	2,58 ± 0,21	BCD	a	2,10 ± 0,23	E	b	5,00 ± 0,20	E	b	1,77 ± 0,21
L1	Microgreen	1,85 ± 0,22	D	b	3,11 ± 0,29	AB	a	11,33 ± 2,65	BCD	a	2,93 ± 0,28
	Baby	1,18 ± 0,15	CD	c	1,44 ± 0,19	DE	b	3,31 ± 0,43	BC	c	1,18 ± 0,47
	Adult	2,35 ± 0,31	DE	a	2,74 ± 0,28	B	a	7,43 ± 1,13	B	b	1,48 ± 0,16
L2	Microgreen	2,29 ± 0,10	BC	b	3,33 ± 0,32	AB	a	12,02 ± 0,78	ABC	a	3,47 ± 0,08
	Baby	1,67 ± 0,03	A	c	2,10 ± 0,15	A	c	3,82 ± 0,68	B	c	1,49 ± 0,14
	Adult	2,49 ± 0,17	CDE	a	2,73 ± 0,15	B	b	6,58 ± 0,30	C	b	1,34 ± 0,04
L3	Microgreen	1,94 ± 0,20	D	a	2,52 ± 0,14	DE	b	13,18 ± 0,22	A	a	3,29 ± 0,14
	Baby	1,46 ± 0,07	AB	b	1,75 ± 0,02	BC	c	3,25 ± 0,38	BC	c	1,08 ± 0,05
	Adult	2,20 ± 0,16	EF	a	3,30 ± 0,14	A	a	6,57 ± 0,62	BC	b	1,45 ± 0,16
L5	Microgreen	1,97 ± 0,19	D	b	2,59 ± 0,14	D	a	10,12 ± 0,21	DE	a	4,18 ± 0,81
	Baby	1,42 ± 0,03	B	c	1,52 ± 0,04	CDE	b	3,08 ± 0,12	C	c	1,31 ± 0,06
	Adult	2,62 ± 0,29	BCD	a	2,37 ± 0,29	CDE	a	6,66 ± 0,44	BC	b	1,58 ± 0,13
L10	Microgreen	1,87 ± 0,30	D	b	2,65 ± 0,07	D	a	12,61 ± 0,98	AB	a	3,20 ± 0,30
	Baby	1,31 ± 0,09	BC	c	1,60 ± 0,02	BCD	b	3,47 ± 0,27	BC	c	1,12 ± 0,07
	Adult	2,39 ± 0,31	CDE	a	2,67 ± 0,10	BC	a	8,49 ± 0,01	A	b	1,37 ± 0,18
L11	Microgreen	1,83 ± 0,15	D	b	1,65 ± 0,20	F	b	11,52 ± 0,78	BCD	a	3,42 ± 0,14
	Baby	1,33 ± 0,14	BC	c	1,34 ± 0,11	E	b	3,89 ± 0,55	B	c	1,17 ± 0,05
	Adult	3,02 ± 0,31	A	a	2,27 ± 0,19	DE	a	6,31 ± 0,30	CD	b	1,51 ± 0,08

Zn (µg g^{-1} DW)			Mn (µg g^{-1} DW)			Si (µg g^{-1} DW)		
CD	a	44,40 ± 2,75	BCD	a	79,70 ± 4,08	C	a	498,02 ± 48,54	BCD	b
CDEF	b	14,04 ± 0,62	BC	b	56,16 ± 3,59	DE	b	129,43 ± 16,96	B	c
CD	b	21,54 ± 2,35	AB	b	42,67 ± 1,69	DE	c	843,17 ± 38,64	CD	a
BC	a	43,66 ± 3,44	CD	a	79,30 ± 1,81	C	a	481,37 ± 42,80	CDE	a
CDE	b	15,41 ± 1,38	AB	c	58,85 ± 8,43	CDE	b	94,40 ± 14,27	CD	b
D	b	22,39 ± 3,33	AB	b	33,73 ± 2,34	F	c	425,07 ± 51,33	EF	a
EF	a	49,08 ± 4,23	B	a	128,19 ± 5,87	A	a	440,36 ± 40,36	DEF	b
A	b	17,23 ± 0,97	A	b	67,61 ± 6,66	AB	b	77,04 ± 8,86	D	c
AB	b	18,99 ± 2,24	BCD	b	44,45 ± 3,54	CD	c	950,57 ± 122,47	BC	a
DEF	a	55,32 ± 6,59	A	a	107,53 ± 0,16	B	a	425,24 ± 51,47	EF	b
AB	b	16,31 ± 2,22	A	b	65,92 ± 5,95	ABC	b	85,86 ± 7,15	CD	c
A	b	19,74 ± 3,13	ABCD	b	42,88 ± 2,13	DE	c	899,95 ± 153,85	BCD	a
A	a	40,62 ± 5,33	D	a	69,48 ± 2,29	D	a	486,24 ± 5,73	CD	a
BC	b	11,51 ± 1,39	DE	b	51,91 ± 4,55	E	b	85,76 ± 10,07	CD	c
B	b	16,71 ± 3,67	D	b	38,07 ± 5,55	EF	c	326,42 ± 55,00	F	b
F	a	38,59 ± 1,26	D	a	78,31 ± 4,21	C	a	451,62 ± 25,97	DEF	b
DEF	b	10,65 ± 1,60	E	c	54,40 ± 6,16	DE	b	134,76 ± 23,18	AB	c
D	b	21,66 ± 1,62	AB	b	49,11 ± 2,27	BC	b	550,57 ± 12,72	E	a
CDE	a	48,41 ± 1,96	BC	a	110,77 ± 9,14	B	a	594,97 ± 46,55	A	b
CD	b	13,20 ± 0,44	CD	c	71,17 ± 1,04	A	b	100,78 ± 12,42	CD	c
D	b	20,35 ± 3,41	ABC	b	41,70 ± 3,06	DE	c	811,80 ± 20,40	CD	a
DEF	a	49,36 ± 2,31	B	a	76,48 ± 1,19	C	a	552,25 ± 35,51	AB	b
F	c	12,15 ± 1,07	CDE	c	60,97 ± 4,81	BCD	b	143,15 ± 4,52	AB	c
CD	b	17,79 ± 2,35	DE	b	60,00 ± 6,00	A	b	816,14 ± 70,52	CD	a
BC	a	41,49 ± 2,27	D	a	79,66 ± 7,76	C	a	523,96 ± 46,68	BC	b
CDEF	b	7,19 ± 0,69	F	c	62,46 ± 8,67	ABCD	b	135,14 ± 26,17	AB	c
C	b	20,08 ± 0,77	ABCD	b	50,51 ± 2,74	B	c	776,72 ± 154,61	D	a
DEF	a	42,93 ± 2,34	D	a	78,62 ± 2,18	C	a	405,00 ± 21,08	F	b
EF	b	11,26 ± 0,51	DE	c	68,60 ± 5,90	AB	b	128,39 ± 6,86	B	c
D	b	23,15 ± 1,10	A	b	50,42 ± 3,46	B	c	1035,12 ± 197,04	B	a
CDEF	a	39,01 ± 0,64	D	a	69,30 ± 1,23	D	a	424,26 ± 28,03	EF	b
DEF	c	16,89 ± 1,06	A	b	69,06 ± 3,71	AB	a	153,32 ± 6,03	A	c
CD	b	20,73 ± 1,27	ABC	b	51,10 ± 2,39	B	b	1980,52 ± 71,43	A	a

*** Sup.Table S1.** The sodium (Na), magnesium (Mg), phosphorus (P), sulphur (S), Zinc (Zn), manganese (Mn) and silicon (Si) concentrations in the collection of the 11 lettuce varieties evaluated in the three development stages (microgreen, baby, adult). Values are the mean±SE of four replicates per variety. The means are subjected to a one-way ANOVA analysis. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ using the LSD test. DW: Dry weight.

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CHAPTER 07

POSTHARVEST CHANGES IN THE NUTRITIONAL PROPERTIES OF COMMERCIAL AND TRADITIONAL LETTUCE VARIETIES IN RELATION WITH OVERALL VISUAL QUALITY

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Abstract

Nowadays the cultivation and consumption of traditional lettuce varieties are growing because they are particularly appealing to consumers for their diversity, and high nutraceutical quality. However, lettuce is a highly perishable product, which results in significant nutritional loss from harvest to final consumers. In this work, the content of some bioactive compounds (chlorophylls, carotenoids, anthocyanins, ascorbic acid, phenols), overall antioxidant capacity and mineral content was monitored in five landraces and four commercial lettuce varieties to compare their variation during the storage period. Visual characterization was done during the postharvest period, as was the determination of the parameters indicative of oxidative stress, to establish the preservation capacity of their physico-chemical attributes. As a general trend, lettuce varieties showed individualized behavior during the postharvest period, which was not necessarily better in the commercial varieties compared to the landraces. Of all the varieties, landrace L10 stood out for not showing excessive variations in its general appearance or nutritional quality throughout its life cycle. However, in terms of initial concentration of bioactive compounds, the reddest varieties (CL4 and L11) stand out. These results indicate variability among varieties, which emphasizes the potential of lettuce landraces in postharvest practices.

Keywords

Antioxidant, bioactive, diversity, landrace, lettuce, nutritional quality, postharvest.

1. INTRODUCTION

In parallel to important crop productivity advances, the food trade has increased, diversified and made product processes complex to reduce postharvest losses, maintain products' valued attributes and guarantee their postharvest quality [1–3]. In this regard, the effect of storage conditions has been widely assessed for leafy vegetables to determine the preservation capacity of physico-chemical attributes [4].

Product quality is a combination of the characteristics, attributes and properties that is valued in human nutrition [5]. Applied to horticultural products, quality can be studied according to four intrinsic components: hygiene-sanitary, technological, organoleptic and nutritional [6]. The last one is the object of our study.

Fresh fruits and vegetables are highly perishable products, and significant quality losses may occur from harvest to final consumers due mainly to two important physiological processes [7] 1) respiration, particularly the rate at which a product breathes. It indicates the metabolic activity of a product's tissues that acts as a useful guide about the length of its commercial life [8,9] 2) transpiration and the loss of moisture with consequent wilting. This is because water is the main component of vegetable products (80-95% of weight, 95% for lettuce) and its loss not only results in a lighter weight, but also in the formation of an unattractive flaccid product of notably less commercial quality [10].

Refrigerated storage is recommended because it slows aging caused by ripening, softening or changes in texture and undesirable metabolic compounds [3,4,8]. Light exposure should also be considered as it may influence products' nutritional balance, especially in terms of nitrate concentration and photosynthetic pigments [11–17].

The biosynthesis, composition and concentration of health-promoting compounds vary widely among leafy crops, and imply the influence of genetic and environmental factors (light and temperature), growing conditions, harvest practices and postharvest handling conditions [18]. Of all leafy vegetables, lettuce (***Lactuca sativa L.)*** is a widely grown and popularly consumed vegetable worldwide, but it is not regarded as nutritional food, primarily due to its high water content (95%) [11]. However, its high content of biologically active compounds, such as vitamins, minerals and organic substances [19,20] makes its nutrient composition the equivalent to other so-called "nutritious" vegetables. In addition, the nutritional and market quality of lettuce relate not only to head size and appearance [17,21], but also to vitamin and mineral contents [22], and to the maintenance of nitrate and nitrite concentrations in leaves at unharmful levels [23,24]. Moreover, leaf color, caused by the balance of chlorophylls, anthocyanins and carotenoids, can also influence the quality of leaves as pigmentation is often associated with the presence of antioxidant compounds [25–28].

Finally, one more, but no less important quality component, appears on the trade market. It takes into account consumer perceptions and is somewhat less tangible for being defined not in terms of intrinsic characteristics, but of consumer satisfaction and behavior on the market [29]. Deterioration in the visual appearance of lettuce leaves generally results in products being rejected consumers [30], which strongly impacts the commercialization chain, especially exportation [3]. The consumer factor is so important for landrace varieties, which are particularly appealing to consumers striving to purchase organic, local and high-quality products, that farmers interest in growing landraces

is growing [31]. Specifically for lettuce, the most appreciated characteristics are the presence of signs of freshness, shiny damage-free leaves, color intensity without yellowing or discoloration, no burns on edges and with ribs that do not crack [4,30,32]. The good aptitude to a certain variety to maintain these attributes during the storage process suggests a clear advantage. It is true that traditional vegetable varieties have been displaced by hybrid varieties from the market, mainly because of their lower yields and inferior pest and disease resistance [31,33]. However, they have also been described as vegetables that well adapt to different conditions [2], besides being considered a reservoir of genetic diversity, particularly for certain attributes of interest, such as their high nutraceutical quality [34]. All this makes landraces a valuable genetic resource to: 1) identify genes of interest for miscellaneous breeding programs; 2) be candidates to be reintroduced into the market after specific studies into nutritional quality, postharvest conservation, among others. Although high genetic diversity exists in the landrace gene pool, this has scarcely been studied and, thus, hinders landrace utilization in agriculture [31].

Therefore, the aim of this work was to evaluate the nutritional quality of lettuce in relation to its different morphological characters by comparing the postharvest evolution in the nutrient composition of five local Valencian lettuce landraces and four commercial varieties to determine the best health-benefit candidates that provide the highest nutritional value and the best bioactive compounds.

2. MATERIALS AND METHODS

2.1. Plant material

Five lettuce landraces and four commercial lettuce varieties ***(Lactuca sativa L.)*** were selected to conduct this study. The selected landraces represent different typologies of the lettuce germplasm collection of Valencia (Spain), while the commercial varieties are among the best-selling lettuces on the market. These local varieties were supplied by the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV, Valencia, Spain) and the Valencian Institute for Agrarian Research (IVIA, Moncada Spain) genebanks. Table 1 provides the abbreviation code, type, numerical code and a brief description of each variety. Figures 1A and 1B complement this table.

2.2. Field Experiment

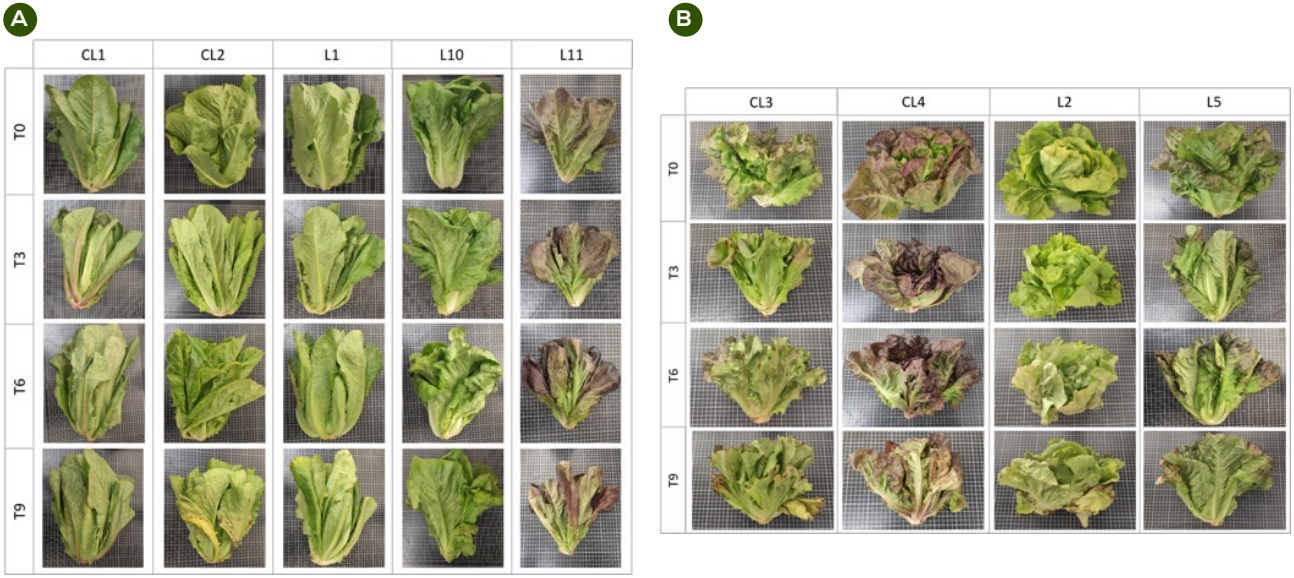
The experiment was done in the experimental facilities of the IVIA in Moncada (Valencia, Spain; 39° 35′ 22.3′′ N, 0°23′ 44.0′′ W, 37 cm above sea level). Seeds were sown in November 2020 in 104-hole trays with 100% natural coconut coir fiber substrate (225 g L-1 density, Cocopeat, Projar Co., 46930 Quart de Poblet, Valencia, Spain) under greenhouse conditions (temperature 21°C, 60% relative humidity (RH) and PAR 1000 µmol m^{-2} s^{-1}).

Seedlings were transplanted under field conditions on December 4, 2020, when plants were 5 cm high, and by ensuring that at least four true leaves had appeared. Each variety consisted in 20 plants distributed into two separate

replicates (10 plants each) with 30-centimeter spacing between each plant and 60-centimeter spacing between each variety. The distance between rows was 100 cm. The soil composition within 20 cm depth was 68% sand, 11% clay and 21% silt (sandy-clay loam), containing 0.61% organic matter, 0.051% total N, less than 8 mg kg−1 of P, 301 mg kg−1 of K and 2.87 meq·100 g−1 of assimilable Mg. Soil electrical conductivity was 0.290 dS m−1 and pH was 8.1. Irrigation met 100% crop evapotranspiration (ETc), as described in Penella et al., [35] performed with a drip system. Nutrients were applied by the irrigation system at a rate (kg ha-1) of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO, and 35 MgO, as recommended by Maroto [36]. The average range of the minimum and maximum temperatures during the field experiment was 1–23 °C for December, -1–26 °C for January, 7–24 °C for February and 6–26 °C for March. Plants were harvested in the adult state on March 23.

2.3. Storage Conditions

Sixteen lettuces were harvested for each variety in the experiment. They were randomly divided into four groups (4 lettuces each) and each subgroup underwent the following storage treatments: samples from the first subgroup (T0) were stored immediately after harvesting without applying any storage treatment. The other subgroups underwent three storage treatments consisting in being left in an industrial refrigeration chamber (Yafri S.L., Alzira, Spain) for 3 days (darkness, 5°C and 98% RH), followed for 0, 3 and 6 days (T3, T6 and T9, respectively) under storage conditions (12-hour photoperiod, 8°C and 88% RH).



*** Figure 1.** Pictures of the nine cultivated lettuce varieties ***(Lactuca sativa L.)*** provided by the Germplasm Banks from COMAV and the IVIA (Spain) at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. The size of the grid cells in the fruit pictures is 1 cm × 1 cm. A: lettuce varieties with no patent head; B: lettuce varieties with a prominent head.

Abbreviation code	Origin	Identification	Plant description
CL1	Commercial	Romaine lettuce long mule ear (Batlle)[a]	Dark green. Elongated shape. Compact and narrow head, barely prominent.
CL2	Commercial	Romaine lettuce from the gardeners (Vilmorín)[a]	Green-yellowish. Elongated shape. Compact and narrow head, barely prominent.
CL3	Commercial	Wonder summer (Batlle)[a]	Green with reddish shades. Remarkable width in relation to height. Compact, rounded and quite prominent head.
CL4	Commercial	Marvel of Four Seasons Butterhead (Batlle)[a]	Dark green with reddish shades; the red is prominent at the edges. Round shape. Quite rounded shape. Full-size head.
L1	Local landrace	BGV005721[b]	Dark green. Pink shades near the principal stem. Elongated shape. Compact and narrow head, barely prominent.
L2	Local landrace	BGV005722[b]	Green-yellowish. Round shape. Full-size head.
L5	Local landrace	BGV005736[b]	Dark green with reddish shades. Elongated shape. Compact and narrow head, quite prominent.
L10	Local landrace	L-10[b]	Dark green. Elongated shape. Compact and narrow head, barely prominent.
L11	Local landrace	L-11[b]	Dark red, almost purple. Remarkable width in relation to height. Head not appreciated.
[a]Commercial name (company), [b]Genbank code			

*** Table 1.** The abbreviation, origin, identification and short phenotypic description of the nine lettuce varieties herein used. Plant material was provided by: (a) a commercial company; (b) the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV, Spain); (c) the Valencian Institute for Agricultural Research (IVIA, Spain).

2.4. Visual Characterization and Weight Loss Determination

In order to visually determine the condition of lettuce plants along the storage chain, the visual quality parameters described by Kader et al. [37] were used with slight modifications as a reference. Firmness, appearance, decay, discoloration, wilting and the appearance of internal lettuce part were the key parameters (Table 2). The same person always carried out this procedure. Visual characterization was performed on each whole lettuce from all the treatments (T0, T3, T6 and T9) prior to sample processing. In parallel, lettuce plants were weighed on the different experiment dates to determine fresh weight (FW) loss during storage.

Trait	Score	Description
Firmness description	1	Soft, easily compressed or spongy
	2	Fairly firm, neither soft nor firm, good head formation
	3	Firm, compact but may yield slightly to moderate pressure
	4	Hard, compact and solid
	5	Extra hard, over-mature, may have cracked mid ribs
Visual quality	1	Extremely poor, disposable
	3	Poor, many defects, limit of salability
	5	Fair, slightly to moderately defects, lower limit of sales appeal
	7	Good, minor defects
	9	Excellent, essentially free from defects
Decay	1	Extreme, disposable
	3	Severe, salvageable but usually not salable
	5	Moderate, objectionable, definitely impairs salability
	7	Slight, sliglhtly objectionable, may impair salability
	9	None
Butt discoloration	1	Extreme, very dark
	3	Severe
	5	Moderate
	7	Slight
	9	None, fresh cut appearance
Wilting	1	Extreme, not acceptable under normal conditions
	3	Severe, definitely objectionable
	5	Moderate, becoming objectionable
	7	Slight, not objectionable
	9	None, fresh cut appearance
Internal part appearance	1	Damage
	2	Objectionable appearance
	3	Good aspect

*** Table 2.** Rating scale for the scoring visual quality of the harvested lettuce varieties.

2.5. Sample Preparation

After each experiment time (T0, T3, T6 and T9), lettuce plants were washed under tap water and dried with paper to remove surface dirt for the nutritional quality determinations. One half of each lettuce was set aside for drying. The other half was chopped and instantly frozen in liquid nitrogen to be stored at -80 °C. The plant material reserved for drying was used for mineral analysis and dry weight (DW) quantification purposes, while the cold-stored samples were used to determine nutraceutical properties. Fresh samples were ground by a mixer mill (MM400, Retsch, Hann, Germany) with liquid nitrogen to prevent melting when processing the samples stored at -80°C. The same machine was used to homogenize the samples dried in a laboratory oven at 65 °C for 72 h, but without adding N_2.

2.6. Nutraceutical Compounds and Antioxidant Capacity

2.6.1 Chlorophyll and Carotenoid Concentration

Total chlorophyll (Chl) and carotenoid (Car) content were determined spectrophotometrically as described by Porra et al. [38] with slight modifications. First, 2.5 mL of 80% acetone (v/v) were added to 60 mg FW of the sample extract to then be centrifuged at 2,000 rpm for 8 min. The supernatant was used for the analysis. Solution absorption was measured at 663.6, 646.6 and 470 nm by a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). Next 80% acetone (v/v) was used as the blank solution. The chlorophyll and carotenoid concentrations of the extracts were calculated by the following equations:

(1) Chl a = 12.25 × Abs663 − 2.55 × Abs648 (μg mL−1)
(2) Chl b = 20.31 × Abs648 − 4.91 × Abs663 (μg mL−1)
(3) Chl a + b = 7.15 × Abs663.6 + 18.71 × Abs646.6 (μg mL−1)(3)
(4) Car = [(1,000 × Abs470 − 1.82 Chl a) − (85.02 × Chl b)]/198 (μg mL−1).

Chlorophylls and carotenoids were expressed as μg g−1 FW.

2.6.2 Anthocyanin Concentration

The total anthocyanin (Ant) content was spectrophotometrically quantified as described by Szepesi et al. [39] with slight modifications. First, 5 mL of methanol:HCl:H_2O solution (90:1:9) were added to 0.1g FW of the homogenized sample previously placed inside glass tubes. Tubes were vortexed and stored in the dark for 1 h at room temperature. Samples were mixed regularly during storage. Then they were centrifuged at 2000 rpm for 5 min and the supernatant was saved for the analysis. Solution absorption was measured at 534, 643 and 661 nm by a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The methanol:HCl: H_2O solution was used as the blank. The anthocyanin concentration of the extracts was calculated by the following formula:

(5) (0.0821 × Abs534 − 0.00687 × Abs643 − 0.002426 × Abs661) × 5 mL/ g FW

Anthocyanin concentration was expressed as μmol g−1 FW.

2.6.3 Ascorbic Acid Concentration

The total ascorbic acid (AsA) was spectrophotometrically quantified as described by Kampfenkel et al. [40]. By adding 1.5 mL of 6% (w/v) trichloroacetic acid (TCA) to 0.2 g FW of each sample. Then samples were centrifuged at

15000 rpm for 5 min at 4°C. The supernatant was saved for further analyses. Next 0.05 mL of the supernatant was mixed with 0.05 mL of 10 mM DTT and 0.1 mL of 0.2 M phosphate buffer (pH 7.4). Samples were incubated in a water bath for 15 min at 42 °C. Afterward, 0.05 mL of 0.5% (w/v) NEM (N-ethylamide) were added and incubated for 1 min at room temperature, before adding 0.25 mL of 10% (w/v) TCA, 0.2 mL of H_3PO_4 4% (w/v), 0.2 mL of 2-2′-dipyridyl, and 0.1 mL of 3% (w/v) $FeCl_3$. Samples were incubated in a water bath for 40 min at 42 °C. Solution absorption was measured at 525 nm by a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The blank solution with no extract was used for calibration purposes. Ascorbic acid was expressed as mg g-1 FW.

2.6.4 Total Phenolic Analysis

The total phenolic (Phe) content was analyzed according to Dewanto et al. [41] with modifications, where 0.1 g FW of the homogenized sample were homogenized in 0.7 mL of 80% (v/v) methanol, vortexed and then incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min. Next samples were centrifuged at 10000 rpm for 15 min at 4 °C and the supernatant was reserved for further analyses. The phenolic concentration was determined by the Folin-Ciocalteau colorimetric method where 20 µL of the supernatant were mixed with 80 µL of methanol and 0.7 mL of Folin-Ciocalteau reagent. This solution was vortexed and incubated in the darks for 5 minutes at room temperature. Then 0.7 mL of $NaHCO_3$ (6%) were added to the solution. The final mix was vortexed and incubated in the darks for 60 min at room temperature. Solution absorption was measured at 765 nm by a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The blank solution with no extract was used for calibration purposes. Each measurement was compared to a standard curve of gallic acid (GA) and the phenolic concentration was expressed as mg of GA equivalent g-1 FW.

2.6.5 Antioxidant Capacity Measurements

Antioxidant capacity (DPPH) was measured following the method reported by Brand-Williams et al. [42] with slight changes. First, 0.1 g FW of the sample were homogenized in 0.7 mL of 80% (v/v) methanol, vortexed and then incubated in an ultrasonic bath (Ultrasonic cleaner, Fungilab, Barcelona, Spain) at medium intensity for 30 min. Next samples were centrifuged for 15 min at 10000 rpm and 4 °C and the supernatant was reserved for further analyses. This was followed by adding 990 µL of 0.065 M of 2,2-diphenyl-1-picrylhydrazyl solution (solved in 80% methanol) to a 20 µL aliquot of the supernatant. Absorbance at 515 nm was measured against a blank solution (80% methanol with no extract) after a 30-minute reaction time at room temperature in the dark by a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). The results were expressed as the percentage reduction of the initial 2,2-diphenyl-1-picrylhydrazyl absorption in extracts.

2.7. Lipid peroxidation

Lipid peroxidation (LP) was determined by the malondialdehyde (MDA) procedure using the thiobarbituric acid (TBA)

reaction according to Heath et al 1968, with slight modifications based on Dhindsa et al [43]. First, 0.1 g FW of the homogenized sample were mixed with 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 5 min at 10000 rpm and 4 °C. The supernatant was recovered. Later 1 mL of supernatant was mixed with 2 mL of the reaction buffer (20% TCA + 0.5% TBA) and samples were incubated in a water bath for 30 min at 95 °C. The non specific background absorbance reading at 600 nm was subtracted from the specific absorbance reading at 532 nm. The results were expressed as nmol 100g-1 FW.

2.8. Hydrogen Peroxide Concentration

Hydrogen peroxide (H_2O_2) content was determined following the method reported by Lopez-Serrano et al. [44], in which 0.25 g FW of were homogenized in 2 mL of 0.1% (w/v) TCA and centrifuged at 10000 rpm for 8 min at 4°C. Then 0.4 mL of the supernatant were diluted with 0.6 mL of 0.1% (w/v) TCA. Afterward, 0.5 mL of 100 µM phosphate buffer and 2 mL of 1M potassium iodide were added to the solution. Absorbance at 390 nm was measured against a blank solution (1 mL of 0.1% (w/v) TCA with no extract) after incubating samples for 1 h at room temperature in the darkness using a spectrophotometer (Lambda 25 UV/VIS, Perkin Elmer, Waltham, USA). Each measurement was compared to a standard H_2O_2 curve and the results were expressed as nmol 100g-1 FW.

2.9. Nitrate Quantification

Nitrate concentration was measured by a basic laboratory meter (Sension+ MM340, Hach, UK) coupled to a nitrate measurement electrode. First, 0.02 g of the DW sample were homogenized by vortexing for 1 min in 8 mL of ionic strength solution (previously prepared with 25 mL of distillated water and a nitrate ionic strength adjustor powder pillow; Hach Permachem, Loveland, USA). Measurements were taken by immersing the electrode in solution while placed on the magnetic stirrer. Equipment calibration was performed with three nitrate standard solutions (Hach, Loveland, USA). The results were expressed as mg g-1 FW.

2.10. Mineral Determination

First of all, 0.1 g of DW sample was burnt in a muffle furnace for 12 h at 550 °C. Macro- and micronutrients were extracted with 5 mL of 2% (v/v) nitric acid in an ultrasonic bath for 30 min at 40 °C. Later 20 mL of 2% nitric acid were added to samples. Mineral concentrations were measured by ICP emission spectrometry (iCAP 6000, Thermo Scientific, Cambridge, UK). This procedure was followed for all the nutrients equally, except nitrates. The results of the macro- and micronutrient concentrations were expressed as mg g-1 FW and µg g-1 FW, respectively.

2.11. Statistical Analysis

The results obtained from this analysis were subjected to a one-way analysis of variance (ANOVA) using Statgraphics Centurion XVII (Statistical Graphics Corporation 2014). The statistical analysis was carried out by taking two different

factors into account: variety type and storage time. The results were expressed as mean±standard deviation. Means were accepted as being significantly different at the 95% confidence interval ($p \leq 0.05$). The mean, maximum and minimum values, coefficient of variation and F-ratio of all the traits were calculated.

A principal component analysis (PCA) was run for the standardized values using pairwise Euclidean distances among accession means to determinate the relations between the genotypes in each development stage. The extracted eigenvalues, and the relative and cumulative proportions of the total variance explained by the principal components (PCs), were calculated. Only those eigenvalues above 1 were considered to be significant. A two-dimensional (2D) scatter plot was prepared (for each storage period) based on a distance matrix for the PCs to visualize the relation that explained the traits.

Four correlation analyses (one for each storage treatment) were also completed of the nutraceutical compounds, DPPH, LP, H_2O_2, NO_3^-, Ca and K concentrations. Individual samples of each accession were subjected to linear regression and correlation coefficients (r) were obtained.

3. RESULTS

3.1. Visual Damage

Table 3 shows quality loss related to the visual description of the harvested lettuces during the storage period. This data is complemented with what is shown in Fig. 1. Visual quality was time-dependent and the lowest values were recorded at T6 and/or T9 depending on the variety. Of all the varieties, CL2, CL3 and L10 obtained better results at the three conservation times, and visual quality was reduced by 10.0±0.8%, 15.7±2.7% and 20.0±1.4% for treatment T3, T6 and T9, respectively. Lettuces L5 and L11 obtained the higher visual damage values from the time the experiment began, with a quality reduction of 24.0±2.5%, 33.9±7.5% and 48.3±11.7% for treatment T3, T6 and T9, respectively.

Variety	Time	Visual quality (%)					Fresh weight (%)				
CL1	T0	100.00			-	-	100.00			-	-
	T3	83.46	±	3.01	C	a	93.82	±	1.40	ABC	a
	T6	78.12	±	3.16	C	b	93.85	±	2.17	A	a
	T9	75.60	±	1.78	B	b	93.34	±	1.08	A	a
CL2	T0	100.00			-	-	100.00			-	-
	T3	89.09	±	3.43	AB	a	94.23	±	1.18	AB	a
	T6	81.95	±	2.43	BC	b	93.11	±	1.22	AB	ab
	T9	79.01	±	2.74	AB	b	91.49	±	1.84	AB	b
CL3	T0	100.00			-	-	100.00			-	-
	T3	90.13	±	3.73	AB	a	92.88	±	0.82	D	a
	T6	87.17	±	1.90	A	ab	90.84	±	1.01	C	b
	T9	81.69	±	5.80	A	b	89.21	±	2.81	B	c
CL4	T0	100.00			-	-	100.00			-	-
	T3	74.43	±	5.04	D	a	91.56	±	2.05	E	a
	T6	69.70	±	6.41	D	a	90.64	±	1.41	C	a
	T9	68.42	±	3.37	C	a	85.54	±	2.19	C	b
L1	T0	100.00			-	-	100.00			-	-
	T3	86.93	±	2.99	BC	a	94.78	±	1.83	A	a
	T6	83.93	±	2.94	AB	a	93.78	±	1.70	A	a
	T9	75.28	±	3.35	B	b	93.96	±	1.30	A	a
L2	T0	100.00			-	-	100.00			-	-
	T3	92.28	±	3.23	A	a	93.10	±	0.88	CD	a
	T6	87.44	±	2.63	A	a	90.40	±	1.89	CD	b
	T9	65.49	±	5.22	C	b	89.09	±	1.88	B	b
L5	T0	100.00			-	-	100.00			-	-
	T3	77.71	±	1.44	D	a	93.29	±	0.91	BCD	a
	T6	71.40	±	1.73	D	b	91.72	±	1.35	C	b
	T9	59.94	±	1.13	D	c	90.59	±	1.44	B	c
L10	T0	100.00			-	-	100.00			-	-
	T3	90.75	±	3.25	AB	a	93.59	±	1.01	BCD	a
	T6	83.76	±	2.11	AB	b	91.92	±	1.37	BCD	b
	T9	79.42	±	5.18	AB	b	90.46	±	1.78	B	b
L11	T0	100.00			-	-	100.00			-	-
	T3	74.19	±	2.83	D	a	93.24	±	0.90	BCD	a
	T6	60.75	±	4.30	E	b	88.97	±	1.66	D	b
	T9	43.39	±	2.92	E	c	83.31	±	2.50	C	c

*** Table 3.** Visual quality (%) and fresh weight (%) of nine harvested lettuce varieties measured at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and storage time, respectively, at $p<0.05$ by the LSD test.

3.2. Fresh Weight Loss

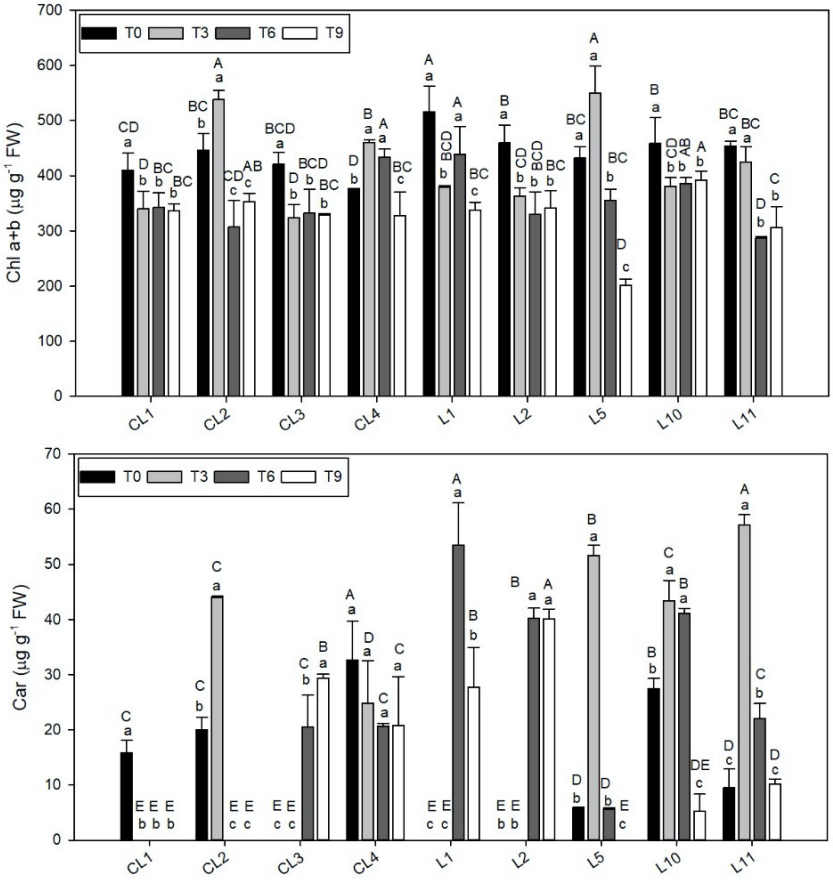
In general terms, the FW percentage (Table 3) lowered by 6.6%, 8.3% and 10.3% for treatment T3, T6 and T9, respectively. The greatest reduction at T3 was for CL4 (8.5%), which decreased by around 10% in two varieties (L2 and L11) at T6. At the end of the experiment (T9), CL4 was once again highlighted for its low FW percentage, as long with L11 (4.2% and 6.4% over the average value for T9, respectively). Two varieties (CL1 and L1) had a high FW percentage

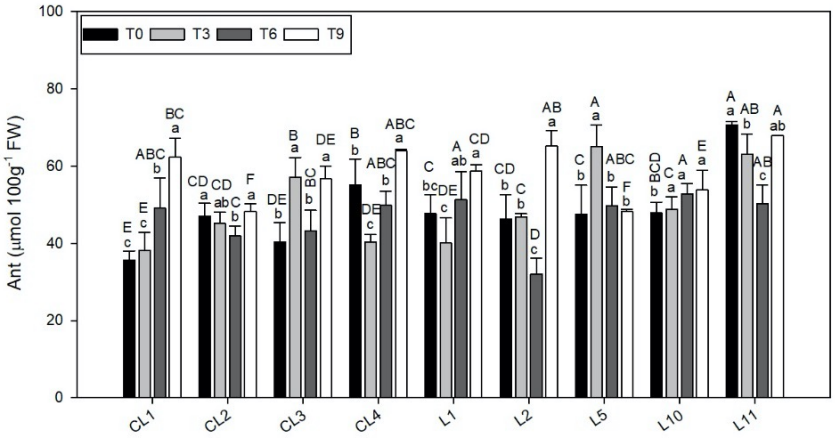
3.3. Nutraceutical Compounds and Antioxidant Capacity

3.3.1 Total Chlorophyll Concentration

The Chl concentration (Fig. 2A, Table 4) was significantly higher at T0 and T3 than for other treatments ($p<0.001$), which highlighted three varieties for their high Chl values: L1 (top level at T0) and CL2 and L5 (top levels at T3). The lowest Chl concentration at T0 went to CL1, CL3 and CL4, while the Chl level at T3 dropped to very low values for five of the nine varieties (CL1, CL3, L1, L2, L10).

At T6 the highest Chl was for CL4, L1 and L10, with the last one also notable at T9 in addition to CL2. At T9, a very low Chl value was obtained for landrace L5.





*** Fig. 2.** The (A) chlorophyll a + b (Chl), (B) carotenoid (Car) and (C) anthocyanin (Ant) concentrations in the nine lettuce varieties evaluated at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under hamber conditions. Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at p<0.05 by the LSD test. FW: Fresh weight.

3.3.2 Total Carotenoid Content

The Car concentration was the most variable trait measured throughout the experiment, reflected by the coefficient of variation at each storage time (between 91.9% and 107.5%; Table 4). In five of the nine varieties (Fig. 2B), the Car concentration was detected only at one storage time or two: CL1 and CL2 (initial time), CL3, L1 and L2 (late time).

A Car concentration was recorded for CL4, L10 and L11 throughout the experiment, but with differences in them. CL4 obtained the stables levels during the whole experiment (medium-high values and without differences between storage times). L10 had good Car levels at T6 and T3, with no significant differences between them, while L11 was highlighted at T3.

L5 was highlighted for having the second highest Car concentration level at T3, despite it being low or absent in the other treatments.

Time	Trait	Unit/Scale	Mean	Range	CV (%)	F-ratio
T0						
	Chl	$\mu g\ g^{-1}FW$	440.83	370.14-564.58***	9.92	5.17
	Car	$\mu g\ g^{-1}FW$	11.53	0.00-39.73***	103.25	39.62
	Ant	$\mu mol\ g^{-1}FW$	48.68	33.40-71.76***	21.52	16.00
	AsA	$mg\ 100g^{-1}\ FW$	35.68	15.78-64.20***	39.09	88.26
	Phe	$mg\ g^{-1}\ FW$	3.84	1.76-9.38***	55.26	41.41
	DPPH	%	17.12	6.47-38.74***	53.71	80.79
	H_2O_2	$nmol\ g^{-1}\ FW$	111.79	8.69-276.61***	77.02	102.63
	LP	$nmol\ 100g^{-1}\ FW$	12.98	9.18-14.88*	9.80	3.23
	NO_3^-	$mg\ g^{-1}FW$	1.47	1.09-2.06*	16.09	3.11
	Ca	$mg\ 100g^{-1}FW$	59.83	39.92-103.45***	29.11	26.30
	K	$mg\ g^{-1}FW$	3.92	3.19-5.03***	12.77	12.22
T3						
	Chl	$\mu g\ g^{-1}FW$	419.17	299.98-650.91***	22.07	11.26
	Car	$\mu g\ g^{-1}FW$	23.54	0.00-58.98***	101.38	250.47
	Ant	$\mu mol\ g^{-1}FW$	49.38	32.32-72.93***	21.00	21.47
	AsA	$mg\ 100g^{-1}\ FW$	28.35	15.45-46.42***	31.74	29.73
	Phe	$mg\ g^{-1}\ FW$	5.71	0.82-12.31***	55.97	41.14
	DPPH	%	19.60	8.11-37.68***	42.84	61.96
	H_2O_2	$nmol\ g^{-1}\ FW$	220.96	76.19-562.95***	72.52	465.14
	LP	$nmol\ 100g^{-1}\ FW$	10.78	7.23-15.52***	16.70	14.12
	NO_3^-	$mg\ g^{-1}FW$	1.39	0.67-2.12***	25.53	6.36
	Ca	$mg\ 100g^{-1}FW$	72.79	43.00-106.77***	25.59	16.74
	K	$mg\ g^{-1}FW$	3.24	1.93-4.29***	16.51	14.84
T6						
	Chl	$\mu g\ g^{-1}FW$	356.12	278.15-470.92***	16.07	7.63
	Car	$\mu g\ g^{-1}FW$	23.58	0-81.43***	91.96	6.37
	Ant	$\mu mol\ g^{-1}FW$	46.75	26.19-61.01***	16.74	6.28
	AsA	$mg\ 100g^{-1}\ FW$	19.46	11.54-28.09***	26.29	11.63
	Phe	$mg\ g^{-1}\ FW$	2.84	1.45-4.71***	34.72	14.14
	DPPH	%	12.03	6.17-22.71***	36.33	39.58
	H_2O_2	$nmol\ g^{-1}\ FW$	164.56	71.36-290.49***	41.73	73.09
	LP	$nmol\ 100g^{-1}\ FW$	10.95	7.55-16.45***	16.65	8.85
	NO_3^-	$mg\ g^{-1}FW$	1.33	0.82-1.87***	19.08	6.51
	Ca	$mg\ 100g^{-1}FW$	65.83	42.92-87.58***	18.82	10.87
	K	$mg\ g^{-1}FW$	2.96	2.28-3.63***	12.40	11.02
T9						
	Chl	$\mu g\ g^{-1}FW$	325.52	190.31-406.94***	15.83	12.35
	Car	$\mu g\ g^{-1}FW$	13.81	0-41.88***	107.50	50.72
	Ant	$\mu mol\ g^{-1}FW$	57.70	46.72-70.34***	12.94	19.00
	AsA	$mg\ 100g^{-1}\ FW$	14.36	6.08-29.73***	38.42	10.36
	Phe	$mg\ g^{-1}\ FW$	5.17	2.13-7.79***	31.23	17.28
	DPPH	%	9.12	4.66-15.77***	33.44	21.46
	H_2O_2	$nmol\ g^{-1}\ FW$	171.26	85.59-280.32***	39.06	95.71
	LP	$nmol\ 100g^{-1}\ FW$	11.89	8.49-17.10***	17.42	10.32
	NO_3^-	$mg\ g^{-1}FW$	1.27	0.29-2.37**	30.46	3.84

*** Table 4.** Variation parameters for the quality traits in the nine harvested lettuce varieties measured at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. Statistics were performed per stage. Values represent the mean, range, coefficient of variation (CV, %), F-ratio and significance (***, **, * indicate significance at $p < 0.001$, $p < 0.01$, $p < 0.05$) for quality traits. Chl: Chlorophylls; Car: Carotenoids; Ant: Anthocyanins; AsA: Ascorbic Acid; Phe: Phenols; DPPH: Antioxidant capacity; H_2O_2: hydrogen peroxide; LP: lipid peroxidation; NO_3^-: nitrate; Ca: Calcium; K: Potassium.

3.3.3 Anthocyanin Concentration

The highest Ant content in the commercial varieties and landraces was shown for storage treatment T9 (mean value between 16.8 and 23.4% higher than the other times; Table 4). Only variety L10 showed no statistical differences between treatments (Fig. 2C) and the Ant concentration in L5 was higher at T3 than in the other treatments.

Of all the varieties, L11 stood out for having the highest Ant concentration in all the treatments. In addition to L11, CL4 also displayed a good Ant level (the second highest value) at T0, while the most remarkable levels at T3 were recorded for CL3, L11, and especially for L5. At T9, the best results were obtained by CL4 and L2 and L11.

On the whole, the lowest Ant concentration was obtained for each storage time by CL2 and CL1, and by L2 at the initial times.

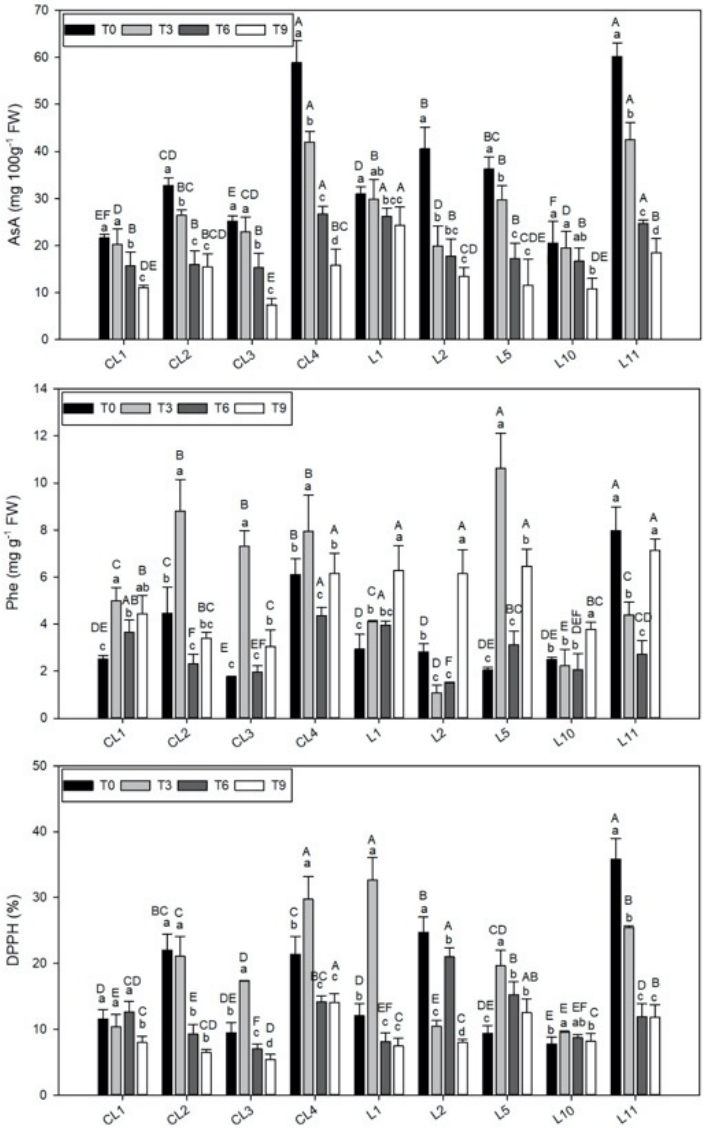
3.3.4 Ascorbic Acid Content

As A content was significantly higher ($p<0.001$) in the initial treatments than at the end of the experiment (nearly 50% reduction; Table 4).

Of all the varieties (Fig. 3A), CL4 and L11 stood out for having the highest AsA concentration in all the treatments (in first place from T0 to T6, with the second highest level at T9). L1 also showed good marks (first place) at T6 and T9. In contrast, CL1, CL3 and L10 had the lowest AsA contents at several storage times.

3.3.5 Total Phenolic Content

Statistically significant differences in Phe content were found in the different storage treatments (Table 4), with the highest mean values recorded at T3 and T9 (5.44±0.38 mg g^{-1} FW, 38.6% higher than the other treatments).



*** Fig. 3.** The (A) ascorbic acid (AsA), (B) phenol (Phe) concentrations and (C) antioxidant capacity (DPPH) in the nine lettuce varieties evaluated at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ by the LSD test. FW: Fresh weight.

At T0, the top Phe concentration was for L11 (107.7% over the T0 average; Fig. 3B). Landrace L5 had the top Phe content at T3 (85.66% over the T3 average) and three commercial lettuces (CL2, CL3 and CL4) also obtained high levels (between 27.9% and 53.9% over the average). At the end of the experiment, four local landraces (L1, L2, L5 and L11) and one commercial variety (CL4) were highlighted for their high Phe concentration (between 7.15 and 6.14 mg g^{-1}FW). In general, the lowest Phe concentration values were for L10.

3.3.6 Antioxidant Capacity

Antioxidant capacity, as determined by the DPPH assay, was statistically higher ($p<0.05$) at T0 and T3 than at T6 and T9 (Table 4).

Of all the varieties (Fig. 3C), CL4 and L11 stood out for their good antioxidant capacities, and L11 was especially relevant at T0 (18.7% over the T0 average) and CL4 at T3 (10.1% over the T3 average). DPPH at L1 and L2 was also remarkable at T0 and T6, respectively (13.1% and 8.9% over the mean, respectively).

On the whole, two commercial varieties (CL1 and CL3) and one local landrace (L10) obtained the lowest results and were especially significant at T0 (between 5.7% and 9.8% below the average) and at T3 for CL1 and L10 (around 9.6% below the average). The lowest DPPH activity value at the end of the experiment was for CL3, and also for CL2 (3.7% and 2.6% lower than the T9 mean, respectively).

3.4. Hydrogen Peroxide

Significant differences ($p<0.05$) were found in the H_2O_2 concentrations among T3, T6 and T9 when related to T0 (97.7%, 47.2% and 53.2% increase, respectively; Table 4). One remarkable finding was that the H_2O_2 content in L11 did not differ between storage times (Fig. 4A) and was similar for three of the four measurements in landraces L2 and L5 (a higher level at T9 and T6, respectively).

At T0, the highest H_2O_2 value was shown by L11 and CL4 (239.1±4.0 nmol g^{-1} FW, 113.8% over the T0 average). CL4 also occupied first place at T3 (150.0% over the T3 average). The H_2O_2 value in L11 was also remarkable at T6 in addition to L5 landrace (255.1±7.3 nmol g^{-1} FW, 61.4% over the T6 average) and L2 was highlighted at T9 (56.2% over the T9 average).

In contrast, CL1, CL3 and L10 had very low H_2O_2 concentrations at T0 (22.8±9.9 nmol g^{-1} FW, 79.6% below the T0 average) and L10 (61.6% decrease) and CL1 (51.4% decrease) at T3 and T6, respectively. The lowest H_2O_2 content at the end of the experiment was noted for three commercial varieties (CL2, CL3 and CL4, 101.1±1.6 nmol g^{-1} FW, 42.4% below the T9 average).

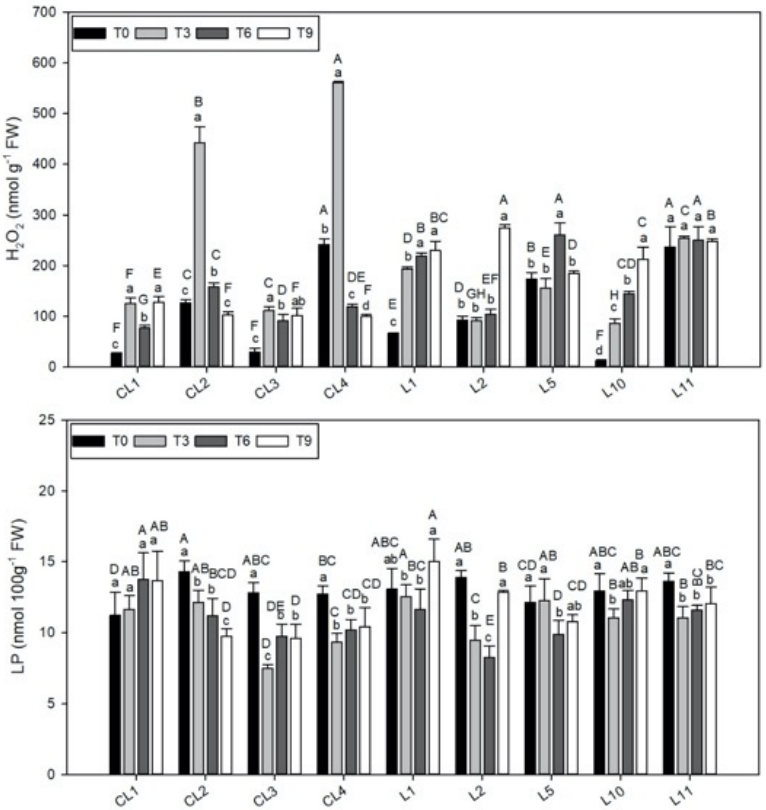
3.5. Lipid Peroxidation

Unlike the H_2O_2 concentration, the highest lipid peroxidation (LP) result appeared at T0 with no significant differences ($p<0.05$) with T9 (Table 4). The LP result in CL1 did not differ between storage times (Fig. 4B), was similar in three

of the four measurements taken in landraces CL4 and L11 (T3, T6 and T9), and the highest level was displayed at T0 in both lettuces.

Of all the landraces, CL1 and L1 had the highest LP results in three of the four storage times, but at T0 and T6, respectively. Conversely, CL3 obtained the lowest values at T3, T6 and T9, but not at T0.

At T0, the most remarkable result was the low LP value in varieties CL1 and L5 (11.7±0.7 nmol 100g-¹ FW, 10.0% below the T0 average), and in CL3 at T3 (30.7% below the T3 average). The lowest LP value at T6 was also recorded at CL3 and L2 (9.0±1.1 nmol 100g-¹ FW, 25.5% below the T6 average). At the end of the experiment, three of the four commercial varieties and one local landrace (CL2, CL3, CL4 and L5) obtained the lowest LP results (10.1±0.5 nmol 100g-¹ FW, 14.8% below the T9 average).



*** Fig. 4** The (A) hydrogen peroxide (H_2O_2) content and (B) lipid peroxidation (LP) in the nine lettuce varieties evaluated at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lowercase letters indicate significant differences between varieties and development stages, respectively, at $p<0.05$ by the LSD test. FW: Fresh weight.

3.6. Nitrate Concentration

Nitrate concentration did not depend on the time exposure to the storage conditions (Tables 4,5), and ranged between 1.47 and 1.27 mg g^{-1} FW from T0 to T9, but with no differences between treatments (P-value: 0.1522). A marked reduction in nitrate concentration was noted only for variety L2 (20.9%, 36.7% and 52.1% for T3, T6 and T9, respectively) in relation to the T0 level (Table 5). Of all the varieties, L11 and CL4 had the highest concentrations (mean values of 1.73 and 1.54 mg g^{-1} FW, respectively), while three commercial lettuces (CL1, CL2, CL3) and two local landraces (L2 and L5) obtained the lowest nitrate concentration (1.23±0.10 mg g^{-1} FW).

Variety	Time	NO_3^- (mg g^{-1} FW)					Ca (mg 100g^{-1} FW)					K (mg g^{-1} FW)				
CL1	T0	1.36	±	0.15	BC	a	48.24	±	5.73	CDE	-	4.07	±	0.38	BC	a
	T3	1.08	±	0.18	DE	b	51.86	±	4.56	D	-	2.79	±	0.06	E	b
	T6	1.25	±	0.04	D	ab	50.67	±	4.54	CD	-	2.70	±	0.10	CD	b
	T9	1.38	±	0.06	B	a	54.05	±	6.82	D	-	2.63	±	0.22	D	b
CL2	T0	1.27	±	0.13	C	-	45.34	±	2.00	DE	b	4.43	±	0.36	B	a
	T3	0.98	±	0.30	E	-	62.35	±	7.75	CD	a	2.91	±	0.06	DE	b
	T6	0.99	±	0.12	E	-	62.21	±	6.43	B	a	3.01	±	0.15	B	b
	T9	1.29	±	0.17	BC	-	54.61	±	5.57	D	a	2.83	±	0.23	CD	b
CL3	T0	1.42	±	0.13	BC	-	54.06	±	6.27	CD	b	3.90	±	0.23	CD	a
	T3	1.38	±	0.34	BCD	-	75.80	±	7.39	B	a	3.29	±	0.32	CD	b
	T6	1.29	±	0.14	D	-	69.12	±	10.12	AB	a	2.97	±	0.28	BC	b
	T9	1.43	±	0.15	B	-	68.38	±	6.35	C	a	2.94	±	0.32	BCD	b
CL4	T0	1.53	±	0.35	BC	-	68.00	±	9.59	B	c	3.63	±	0.30	DE	-
	T3	1.59	±	0.21	ABC	-	93.18	±	13.14	A	ab	3.67	±	0.38	AB	-
	T6	1.56	±	0.22	AB	-	78.38	±	7.94	A	bc	3.33	±	0.22	A	-
	T9	1.47	±	0.09	AB	-	94.83	±	8.86	A	a	3.27	±	0.06	AB	-
L1	T0	1.50	±	0.05	BC	-	42.53	±	3.30	E	c	3.77	±	0.20	CDE	a
	T3	1.48	±	0.07	ABC	-	56.90	±	6.07	D	a	3.35	±	0.22	BC	b
	T6	1.38	±	0.28	BCD	-	48.08	±	6.00	D	bc	2.79	±	0.11	BCD	c
	T9	1.27	±	0.05	BC	-	55.51	±	5.82	D	ab	2.91	±	0.17	BCD	c
L2	T0	1.63	±	0.26	AB	a	73.21	±	11.58	B	b	3.66	±	0.17	DE	a
	T3	1.29	±	0.18	CDE	b	96.64	±	8.29	A	a	3.44	±	0.34	BC	ab
	T6	1.03	±	0.09	E	bc	72.73	±	6.99	A	b	2.59	±	0.30	D	bc
	T9	0.78	±	0.13	E	c	80.28	±	12.78	B	b	2.99	±	0.36	BCD	c
L5	T0	1.35	±	0.07	BC	bc	55.27	±	4.38	C	c	3.57	±	0.18	DE	a
	T3	1.66	±	0.23	AB	a	91.42	±	9.32	A	a	3.96	±	0.32	A	b
	T6	1.51	±	0.03	ABC	ab	77.79	±	6.31	A	b	3.39	±	0.16	A	bc
	T9	1.16	±	0.01	CD	c	80.28	±	4.82	B	b	3.18	±	0.27	ABC	c
L10	T0	1.36	±	0.19	BC	-	54.78	±	6.40	CD	-	3.39	±	0.26	E	a
	T3	1.11	±	0.24	DE	-	53.14	±	8.95	D	-	2.28	±	0.25	F	b
	T6	1.31	±	0.15	CD	-	59.44	±	6.79	BC	-	2.53	±	0.26	D	b
	T9	1.02	±	0.27	D	-	56.08	±	7.67	D	-	2.17	±	0.13	E	b
L11	T0	1.82	±	0.22	A	-	97.02	±	5.39	A	a	4.84	±	0.20	A	a
	T3	1.80	±	0.05	A	-	73.80	±	9.94	BC	b	3.45	±	0.18	BC	b
	T6	1.63	±	0.11	A	-	74.01	±	5.12	A	b	3.35	±	0.13	A	b
	T9	1.67	±	0.18	A	-	95.43	±	10.02	A	a	3.42	±	0.34	A	b

*** Table 5.** Variation parameters for the quality traits in the nine harvested lettuce varieties measured at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. Values are the mean±SE of four replicates per landrace. The mean is subjected to a one-way ANOVA. Different capital and lower case letters indicate significant differences between varieties and storage time, respectively, at $p<0.05$ by the LSD test. . Chl: Chlorophylls; Car: Carotenoids; Ant: Anthocyanins; Asa: Ascorbic Acid; Phe: Phenols; DPPH: Antioxidant capacity; H_2O_2: hydrogen peroxide; LP: lipid peroxidation; NO_3^-: nitrate; Ca: Calcium; K: Potassium.

3.7. Mineral Concentration

Nitrate concentration did not depend on the time exposure to the storage conditions (Tables 4,5), and ranged between 1.47 and 1.27 mg g^{-1} FW from T0 to T9, but with no differences between treatments (P-value: 0.1522). A marked reduction in nitrate concentration was noted only for variety L2 (20.9%, 36.7% and 52.1% for T3, T6 and T9, respectively) in relation to the T0 level (Table 5). Of all the varieties, L11 and CL4 had the highest concentrations (mean values of 1.73 and 1.54 mg g^{-1} FW, respectively), while three commercial lettuces (CL1, CL2, CL3) and two local landraces (L2 and L5) obtained the lowest nitrate concentration (1.23±0.10 mg g^{-1} FW).
Table 5 presents the concentration of two selected macronutrients (Ca and K). The other minerals are presented in Supplementary Table S1.

Storage treatments did not statistically influence Ca concentration (p-value: 0.3306), but significant differences in lettuces were found ($p<0.001$). Two of the nine varieties (CL1 and L10) had a similar Ca concentration at each time. Varieties CL4, L2 and L11 had the top Ca concentration levels (first or second place) at each storage time, which was a general trend, followed by L5 from T3 to T9 (between 15.0% and 32.7% higher than the average value at T0, T3, T6 and T9). In contrast, CL1, CL2 and L1 presented the lowest values (between 18.5% and 24.2% lower than the average value).

For K concentration (Table 4), statistic differences were found between storage treatments ($p<0.001$) because all the landraces except CL4 presented the highest K level at T0. At first treatment, landrace L11 was highlighted for its high K concentration (4.8 mg g^{-1} FW, 23.6% higher than the T0 average, Table 5), with its maximum at T3 in CL4 and L5 (13.4% and 22.4% above the T3 average, respectively). CL4, L5 and L11 had a high K level for both T6 and T9 treatments (13.4% and 12.3% above the T6 and T9 average, respectively).

3.8. PCA Analysis

The PCA and those eigenvalues above 1 reflected a different pattern in the correlation of the lettuces in the four treatments (Table 6).

T0	First PC	Second PC
Chl		0.628
Car		-0.562
Ant	0.371	
AsA	0.372	
Phe	0.375	
DPPH	0.376	
H_2O_2	0.333	-0.235
LP	0.198	0.352
NO_3^-	0.327	0.207
Ca	0.351	
K	0.243	
Eigenvalue	6.21	1.97
Variance explained (%)	56.46	17.89
Cumulative variance explained (%)	56.46	74.35

T3	First PC	Second PC	Third PC	Fourth PC
Chl	0.334	0.354	0.195	-0.290
Car	0.255	0.317	0.430	
Ant	0.212	-0.197	0.605	
AsA	0.400		-0.216	0.291
Phe	0.301	0.172		-0.466
DPPH	0.332		-0.408	0.304
H_2O_2	0.286	0.255	-0.417	-0.274
LP		0.518		0.332
NO_3^-	0.357	-0.302		0.417
Ca	0.268	-0.417		-0.362
K	0.366	-0.318		
Eigenvalue	4.63	2.18	1.69	1.10
Variance explained (%)	42.11	19.85	15.35	10.01
Cumulative variance explained (%)	42.11	61.96	77.31	87.32

T6	First PC	Second PC	Third PC	Fourth PC
Chl	0.283	-0.202	-0.402	-0.289
Car		-0.189	-0.601	0.429
Ant	0.440	-0.169	0.225	
AsA	0.377		-0.371	
Phe	0.415			-0.511
DPPH		0.345	-0.282	-0.391
H_2O_2	0.301	0.236		0.547
LP	0.178	-0.402	0.400	
NO_3^-	0.446	0.163		
Ca		0.560		
K	0.261	0.459	0.212	
Eigenvalue	3.81	2.91	1.86	1.04
Variance explained (%)	34.66	26.47	16.88	9.43
Cumulative variance explained (%)	34.66	61.13	78.01	87.44

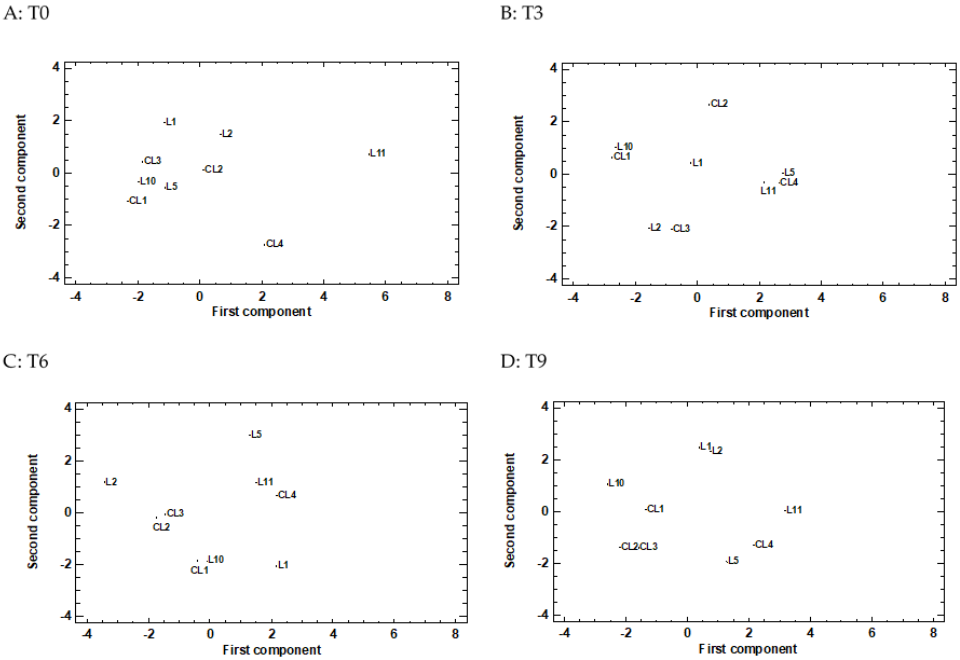
T9	First PC	Second PC	Third PC	Fourth PC
Chl	-0.263	0.295	-0.444	0.159
Car		0.304	-0.445	-0.463
Ant	0.280	0.264	-0.473	
AsA	0.233	0.277		0.495
Phe	0.456	0.157	0.225	
DPPH	0.387	-0.181	0.203	
H_2O_2	0.184	0.452	0.294	-0.173
LP		0.522	0.191	0.281
NO_3^-	0.162	-0.290	-0.354	0.573
Ca	0.430			-0.265
K	0.428	-0.186		
Eigenvalue	4.12	2.74	1.38	1.24
Variance explained (%)	37.49	24.89	12.59	11.26
Cumulative variance explained (%)	37.49	62.38	74.97	86.23

*** Table 6.** The principal component analysis (PCA) of the nine lettuce varieties based on the quality traits represented in the two first components (PC 1 X-axis; PC 2, Y-axis) of the PCA for A) 0 days (T0) (56.46% and 17.89% of total variation, respectively), 3 days (T3) (42.11% and 19.85% of total variation, respectively), 6 days (T6) (34.66% and 26.47% of total variation, respectively) and 9 days (T9) (37.49% and 24.89% of total variation, respectively) after conservation under chamber conditions..

At T0, the number of the most significant PCs was that the first and second PCs accounted for around 74% (56.5% and 17.9% for the first and second PCs, respectively) of the total variation for the studied traits. The first PC positively correlated with all the traits in seven of the 11 parameters (NO_3^-, Ca, Ant, AsA, Phe, DPPH, H_2O_2). The correlation values lay between 0.33 and 0.38. The second PC displayed two marked correlations, one was positive with Chl

(0.63) and one was negative with Car (-0.56). From the projection on the PCA plot (Fig. 5A), a group of a large number of varieties appeared (7 out of 9) located in the central-left zone of the graphic, while two varieties were separately located. L11 was the variety furthest to the right from the rest because it had top levels for four traits: Ant, AsA, Phe and DPPH (Figs. 2C and 3A-C). The top AsA level and the second highest Phe concentration (Figs. 3A,B) left variety CL4 slightly to the right, and further to the bottom of the plot for its top Car and H_2O_2 concentrations (Fig. 2B and Fig. 4A, respectively).

At T3, T6 and T9 four PCs were recorded whose eigenvalue exceeded the unit and described around 87% of the variability among varieties for the three storage times. The distribution of lettuces in the PCA was located mostly by the variability of the traits in the first PC (between 42% and 35%). Most correlations were positive, and the highest coefficients were related to some mineral content (NO_3^- and K for T3, NO_3^- for T6, and both Ca and K for T9), pigments at T3 and T6 (Chl and Ant, respectively), AsA at T3 and T6 and DPPH at T3 and T9. The Phe concentration was notable at the three storage times, and the correlation value increased (from 0.30 to 0.46) as the experiment continued. The second PC explained between 20% and 26% of the distance between landraces and the most important correlation was recorded for the LP trait (0.52, -0.40 and 0.52, at T3, T6 and T9, respectively). The other remarkable correlations in the second PC were related to the three minerals (negative) and Chl (positive) at T3, Ca, K and DPPH at T6 (positive) and the H_2O_2 concentration at T9 (positive).



*** Fig. 5.** The principal component analysis (PCA) of the nine lettuce varieties based on the quality traits represented in the two first components (PC 1, X-axis; PC 2, Y-axis) of the PCA for A) 0 days (T0) (56.46% and 17.89% of total variation, respectively), 3 days (T3) (42.11% and 19.85% of total variation, respectively), 6 days (T6) (34.66% and 26.47% of total variation, respectively) and 9 days (T9) (37.49% and 24.89% of total variation, respectively) after conservation under chamber conditions.

Although the projection on the PCA plot (Fig. 5B-D) spread the nine varieties over the whole area, some lettuces remained together along the three graphics. This was the case of CL4, L5 and L11 (central-right area) because they had similar Ca and K concentrations (top levels) for the three storage times. The good AsA levels at T3 and T6, and Phe and DPPH at T9, also favored their proximity at each particular time. Lettuces CL1 and L10 were very close (central-left area) for their low minerals contents, and good AsA and DPPH activity throughout the experiment. The top Chl level and the presence of Car in L10 at T9 placed this variety slightly further up and further left than CL1.

The CL2 and CL3 pair separated at T3, but moved closer as the experiment continued given their low Phe and DPPH levels at T6 and T9 (two of the four varieties with the lowest results for these traits at later times).

L1 and L2 were also very distant at T3, and especially so at T6, for their different Phe and H_2O_2 concentrations (higher in L1 than L2), but they moved closer at T9 because the Phe concentration reached top levels in L2, and also for their similar DPPH activity. In addition, these two local landraces were placed at the top of the graphic for their remarkable LP and H_2O_2 concentrations.

3.9. Correlation Between Quality Compounds

Correlation analyses were carried out to estimate the relation between the top quality traits at the four storage times (Table 7).

At T0, all the statistically significant pairwise coefficients (28 pairs of traits of the 55 studied ones) showed a positive correlation. Of them, 17 showed strong positive relations ($p<0.001$), with the most representative in DPPH vs. the three minerals and vs. Ant, AsA and Phe (r between 0.806 and 0.545). The multiple combinations among Phe, Ant and AsA in addition to the Ca concentration were also closely related, with H_2O_2 vs. Ant, AsA, Phe and DPPH. LP was positive, but barely related to the Ant concentration and DPPH capacity.

T0	Chl	Car	Ant	AsA	Phe	DPPH	H_2O_2	LP	NO_3^-	Ca	K
Chl		-0.348	0.193	-0.037	-0.124	0.072	-0.091	0.311	-0.089	-0.058	0.025
Car			0.189	0.095	0.378*	0.068	0.252	-0.117	-0.167	0.044	0.016
Ant				0.748***	0.767***	0.710***	0.676***	0.385*	0.477**	0.738***	0.449**
AsA					0.829***	0.779***	0.927***	0.229	0.465**	0.744***	0.329
Phe						0.806***	0.815***	0.216	0.409*	0.668***	0.539**
DPPH							0.654***	0.410*	0.545***	0.739***	0.626***
H_2O_2								0.224	0.211	0.571**	0.263
LP									0.121	0.188	0.160
NO_3^-										0.645***	0.340*
Ca											0.388*
K											

T3	Chl	Car	Ant	AsA	Phe	DPPH	H_2O_2	LP	NO_3^-	Ca	K
Chl		-0.739***	0.742***	0.660***	-0.744***	0.640***	0.528**	0.810***	-0.745***	0.740***	-0.811***
Car			-0.726***	-0.491**	0.982***	-0.561***	-0.253	-0.715***	0.872***	-0.763***	0.966***
Ant				0.306	-0.750***	0.349*	0.079	0.532***	-0.732***	0.745***	-0.770***
AsA					-0.421*	0.832***	0.761***	0.417*	-0.484**	0.601***	-0.523**
Phe						-0.500**	-0.217	-0.695***	0.997***	-0.713***	0.986***
DPPH							0.649***	0.562***	-0.554***	0.528***	-0.590***
H_2O_2								0.299	-0.277	0.378*	-0.302
LP									-0.682***	0.490**	-0.744***
NO_3^-										0.725***	0.965***
Ca											-0.770***
K											

T6	Chl	Car	Ant	AsA	Phe	DPPH	H_2O_2	LP	NO_3^-	Ca	K
Chl		0.408*	0.250	0.356	0.515**	-0.097	0.037	0.262	0,208	-0.138	-0.025
Car			-0.010	0.486**	-0.172	0.002	0.084	-0.159	0,054	-0.134	-0.352
Ant				0.293	0.344	-0.367*	0.442*	0.378*	0.545**	-0.068	0.265
AsA					0.465**	0.021	0.327	-0.062	0.481**	0.010	0.322
Phe						-0.076	0.087	0.340	0.502**	-0.085	0.353*
DPPH							0.041	-0.430**	-0,074	0.413*	0.032
H_2O_2								0.001	0.464*	0.294	0.530**
LP									0,137	-0.522**	-0.186
NO_3^-										0,327	0.652***
Ca											0.668***
K											

T9	Chl	Car	Ant	AsA	Phe	DPPH	H_2O_2	LP	NO_3^-	Ca	K
Chl		0.178	0.124	0.089	-0.257	-0.324	-0.020	0.297	-0,128	-0.353	-0.431*
Car			0.498**	0.324	0.113	-0.205	0,355	0.278	-0,285	0.124	0.189
Ant				0,241	0.402*	0,158	0.338	0.423*	0,152	0.445*	0.290
AsA					0.519**	0.208	0.396*	0.398*	0,051	0.064	0.212
Phe						0.676***	0.527**	0.291	0,177	0.584***	0.543***
DPPH							0.155	-0.126	0,218	0.669***	0.443**
H_2O_2								0.547**	-0.407*	0.153	0.054
LP									-0,223	-0.252	-0.216
NO_3^-										0.374*	0.536**
Ca											0.779***.
K											

*** Table 7.** Linear correlation coefficient (r) and its significance among the quality traits in the nine harvested lettuce varieties measured at 0 (T0), 3 (T3), 6 (T6) and 9 (T9) days after conservation under chamber conditions. ***, **, * indicate significance at p<0.001, p<0.01, p<0.05 for r. Chl: Chlorophylls; Car: Carotenoids; Ant: Anthocyanins; Asa: Ascorbic Acid; Phe: Phenols; DPPH: Antioxidant capacity; H_2O_2: hydrogen peroxide; LP: lipid peroxidation; NO_3^-: nitrate; Ca: Calcium; K: Potassium.

T3 was the storage time with the most correlations (23 positive traits and 24 negative ones) and most were strongly correlated (80.8% with a p-value<0.001). The most remarkable positive relations were the combinations among the Phe, Car, NO_3^- and K concentrations (r value between 0.872 and 0.997), and between Ca, Chl, Ant and AsA (r value between 0.601 and 0.742). DPPH was positive and strongly correlated with the Ca, Chl and AsA concentrations in addition to H_2O_2 and LP. Strong and negative relations appeared between both Chl and Ant pigments vs. several traits (Car, Phe, NO_3^- and K). The negative, but more moderate trend, was between the same four parameters and DPPH and LP.

At T6, the number of positive correlations lowered to 15 and the strongest coefficients were observed only in two pairwise coefficients (NO_3^- vs. K and Ca vs. K). Moderate/low positive correlations were found among NO_3 and Ant, AsA, Phe and H_2O_2. Similar results were obtained between the Car and Chl pigments and the Car vs. the AsA concentration. H_2O_2 and LP were positively related to the Ant concentration. Only three negative and moderate/low relations were observed at T6: LP vs. the Ca concentration and vs. DPPH capacity, in addition to DPPH vs. Ant.

At the end of the experiment at T9, the pairwise coefficients showed positive correlations in 17 of the 55 combinations, with the strongest ones among the combinations of Ca, K, Phe and DPPH activity. Moderate positive relations were recorded between the combinations of minerals (NO_3^-, Ca and K), in addition to LP vs. three traits (H_2O_2, Ant, Phe) and H_2O_2 vs. AsA and the Phe concentrations. Only two negative relations appeared between the pairs NO_3^- vs. H_2O_2 and K vs. Chl.

4. DISCUSSION

Postharvest environmental conditions, particularly temperature, RH and light, have a major impact on overall fruit and vegetable quality [3,17]. Although lettuce is not generally stored for long periods of time, its quality can be maintained for about 15 days at 0 °C with minimum 95% RH [45]. Likewise, it is known that postharvest decay in lettuce and other vegetable crops is a major source of financial loss for producers [46] and ranges from about 20% to 40% of all economic profits [2]. Harvested products are metabolically active, and undergo ripening and senescence processes that must be controlled to prolong postharvest quality [3].

Loss of product quality during shelf life is usually first perceived as declining visual appearance [8]. Lettuce is a highly perishable vegetable whose quality and shelf life are principally limited by dehydration. Cellular wall degradation as a consequence of turgidity loss [4] affects texture, firmness and color by producing detrimental texture changes and enzymatic browning during postharvest storage [3,4,8]. For this matter, controlling the tissue water status is crucial for lettuce quality, whose content in water exceeds 95% [11]. To prevent excessive water loss, vegetables need to be stored below optimum humidity level, generally at 95% to 98% RH [4]. For this reason, these moisture levels were maintained throughout our trial, although lettuce weight loss is unavoidable during storage.

Leaf anatomy, including cell wall thickness and strength, cell size and cell:cell adhesion, determines firmness, along with leaf turgor as established by water content [47]. Camejo et al. [17] suggested making readjustments to the Ca

content promoted by a specific light condition, which could modulate the rigid cell wall and, consequently, leaf texture. In our study, the variety with the most Ca accumulation was landrace L11, followed by L2 and CL4. This finding could indicate that they were better capable to absorb or accumulate this cation, likely through more efficient Ca acquisition or transport systems. However, L11 was the most affected variety by storage in visual appearance terms. It is also true that during storage, Ca seemed to be related to the analyzed antioxidant compounds, which suggests that varieties with a high Ca content are also those with a better antioxidant response, which was the case of L11. However, leaf texture and overall visual quality are factors that influence lettuce's market value and its consumer appeal [8,17]. Among varieties, CL1 and L1 could be the most attractive for consumers because these varieties suffered the least losses in wilting and FW terms, although their Ca content was unremarkable.

Another major visual quality indicator is the retention of green color, associated with chlorophyll content [4]. Lettuce discoloration is unpredictable and, therefore, difficult for growers and retailers to manage. One key approach to reduce the discoloration risk would be to breed cultivars that are 'resistant' to postharvest discoloration development [30]. In this context, landraces can be considered potential sources. In our study, all except variety L11, we visually observed that the other landraces behaved similarly to the commercial varieties in discoloration terms (a drop of around 20% from T0 to T9; Table S1). This implies that their storage capacity would be the equivalent, but they are not presently considered for market purposes. Chlorophyll loss was observed in all the varieties, except CL2, CL4 and L5 whose chlorophyll content increased at the beginning of storage times. As chlorophyll collaborates in neutralizing free radicals from damaging healthy cells [48], increased Chl amount could be related to the stress protective response of these varieties. Along the same line, the visually observed discoloration could be associated with the generalized Chl loss that took place among varieties. Thus the differences in the changes in discoloration overs time appeared to be specific to lettuce type, as proposed by Atkinson et al. [30]. Examples of this would be the discoloration scores obtained for some landraces compared to the commercial varieties with a similar structure, such as L5 vs. CL3 (semi-open head) and L1 and L10 vs. CL2 (romaine type).

Similarly, chlorophylls (green color) are not the only pigment relevant for color determination because the pigments that cause coloration in lettuce leaves also include anthocyanins (red-purple color) and carotenoids (yellow-orange color) [22]. The wide range of varieties available on the market includes different colors or mixtures in leaf zones or in the plant itself. So the proportion of these compounds in lettuce is very interesting, and even more so for its nutraceutical value.
In photosynthetic tissue, carotenoids, together with chlorophylls, operate in light harvesting and perform tasks during photo-protection by quenching free radicals, singlet oxygen and other reactive oxygen species (ROS) [49]. At the different storage times of our study, there were significant correlations between carotenes and other antioxidant compounds, which would highlight the antioxidant properties of these pigments in lettuce. The biosynthesis of carotenoids is regulated by light, which means that differences in carotenoid content in lettuce types has been suggested to be related to head structure [11,12]. Kim et al. [11] and Baslam et al. [13] suggested that as crisphead has a closed head, the leaves inside receive less light than the leaves in open-head or semi-open lettuce types, which results in lower carotenoid synthesis. In contrast, romaine lettuce has an open-head structure which allows more light to penetrate and results in higher carotenoids accumulation. However, these assumptions were not met in our study. Variety CL4, one which has the largest and closest heads, obtained the highest Car values at T0, while Car in the L1

and L2 romaine lettuces was not even detected. Simko et al. [22] stated that the yellow-orange color provided by carotenes was masked in photosynthetically active tissues. It was most likely that the large amount of Chl in lettuce tissues could have eclipsed Car content because both compounds were determined together. Specifically, landrace L1 had the highest Chl concentration in our study at T0. When observing the Car content over the postharvest period, no pattern appeared to explain the behavior of varieties, not even for lettuce type. Changes in the content of these pigments seemed to be depend on each variety. CL3, L1 and L2 started to synthesize carotenes from day 3, when lettuces were exposed to light. In these cases, the relation of carotenes with the incidence of light was evident. However, varieties CL1 and CL2 displayed the opposite behavior. For all these reasons, Car content did not provide us with any enlightening information for our study. We can only state that Car concentration appears to be specific to lettuce variety, which is most likely because of genetic background.

As mentioned in the previous case, the Ant concentration also seemed limited partly by the incidence of light [16], favored by low leaf overlap rates. Anthocyanins appear abundantly in red-colored lettuce [50,51]. The biological functions of anthocyanins have been related to their antioxidant capacity and as photo-protectors of the photosynthetic apparatus [28,52]. This would, therefore, imply that the reddest varieties, with the highest Ant concentration, namely CL4 and L11, were those with the highest antioxidant potential. We observed that the correlations between anthocyanins and the other antioxidant compounds in this study were less correlated as postharvest time elapsed, but the relation linking this pigment to total phenols and overall antioxidant capacity remained significant and positive. We also observed that Ant content tended to increase during storage, which led us to believe that its antioxidant capacity was key during the postharvest period. So those varieties with a good synthesis capacity of anthocyanins would be interesting. Variety L10 should also be highlighted in Ant concentration terms. Although it did not stand out for its synthesis of anthocyanins during storage, it remained unperturbed. That is to say, it would appear that storage conditions did not destabilize it as anthocyanins were neither significantly degraded nor synthesized. Likewise, there is evidence for a high heritability of Car, Chl [53] and Ant [54] contents that would facilitate the selection of new cultivars with a desirable combination of traits for breeding programs [22].

Regarding Phe content, it has been suggested that red leaf lettuce cultivars present larger amounts of phenolics [8,11,25,27,50] which is, in turn, related to their high anthocyanin content and, thus, contribute to total antioxidant capacity [27]. This would allow us to think that the behavior of phenols during lettuce preservation should follow a similar pattern to that of anthocyanins. It is true that reddish varieties L11 and CL4 stood out for their high Phe contents. As with anthocyanins, during storage a generalized phenol synthesis event was observed throughout the postharvest period. The individual behavior of each variety did not exactly coincide but was similar as far as the compound synthesis time is concerned. In the specific case of Phe, phenolic content increased at T9 in all the studied varieties, even in variety L10, which had remained unchanged until that time. This reflects the close relation between phenols and anthocyanins, which was also supported by the positive correlations between both at T0 and T9. At intermediate times T3 and T6, a positive correlation between the two compounds was not detected due to the diversity of responses obtained by varieties, although the majority of varieties showed obvious changes in Phe content at T3. The results obtained with this trial contrast those reported by Ferreres et al. [55] and Dupont, et al. [56] who claimed that postharvest processing and storage resulted in significant losses of flavonoids and phenolic contents in several lettuce cultivars, which could be due to either the employed storage conditions or the resistance capacity

of the studied varieties.

As with Car and Ant, there is evidence that light intensity plays a crucial role in AsA content in cultivated leafy vegetables [17,23,24]. Increased light intensity promotes the activity of the enzymes involved in vitamin C metabolism, which accelerates its synthesis in plant leaves [57]. Ascorbic acid, also known as vitamin C, is considered a primary source of antioxidants in human diet because it reduces plant oxidative processes [58]. As previously mentioned, red varieties CL4 and L11 contained the largest amount of AsA, followed by varieties L2 and L5. In our varieties, this indicates that plant structure, in relation to light incidence, does not particularly influence vitamin synthesis as varities CL4 and L2 were those with the most pronounced bud. Therefore in this particular case, the genetic compound that would regulate vitamin C synthesis would be stronger than the environmental one. While observing the behavior of varieties, we observed that AsA content decreased with time, and became more gradual (with no significant differences in the initial treatments) in varieties CL1 and L1. For variety L10, there was no significant loss of AsA until T9. Once again, this highlights the landrace's conservation capacity. All this supports the notion that the postharvest decay of fruit and vegetables is due mainly to the continuous consumption of their own nutrients [59,60]. For this reason, when we observe the correlation table, we can see that AsA is closely related to the total antioxidant capacity of lettuce at T0, but this relation subsequently fades.

H_2O_2 content and LP were determined as oxidative stress indicators. It has been suggested that anthocyanins inhibit LP [61,62]. This prediction is true when we look at the results of Ant and LP because each LP spike matched an anthocyanin synthesis event, with its subsequent peroxidation rate correction. A permanent correlation also appears between Ant content and LP, which was accentuated at T3. Based on this finding, the varieties with higher Ant content or greater pigment synthesis capacity under stress would better control the LP rate which, in our case, would probably be CL4, L5 and L11. LP was not excessively altered in any variety but was unlike that observed in H_2O_2 quantification. A gradual increase in H_2O_2 content took place mainly in varieties CL1, L1 and L10. Conversely to this event, variety L11 remained unperturbed, and varieties CL2, CL4 and L5 were able to effectively slow down the rising H_2O_2. These could indicate that other types of ROS could be implicated in the LP process, an apparently controlled process on a generalized basis, while the system controlling H_2O_2 concentration would be significantly altered. This could indicate the lesser ability of varieties CL1, L1 and L10 to withstand postharvest conditions. However, the other quantifications and the performed visual quality determination indicated the opposite. Therefore, the imbalance in the H_2O_2 accumulation in these cases may not cause these varieties excessive damage.

Concerning nitrate accumulation, nitrogen intake is known to influence plant growth and development [63], and its main usable forms are NO_3^- and ammonium (NH_4^+) [64]. The nitric form is preferred by most plants [65], including lettuce [66,67]. Unfortunately, nitrate content is considered to be potentially dangerous for health, especially for its reaction products and metabolites, such as nitrite, nitric oxide and N-nitroso compounds [17,68,69]. Therefore, in line with the World Health Organization, European Commission Regulation 1881/2006 (EC, 2006) sets the maximum thresholds of nitrates in lettuce. According to this regulation, fresh lettuces harvested between October and March, and grown in the open air, must not exceed a threshold of 4000 mg NO3 kg^{-1} FW, except for 'Iceberg' type (2000 mg NO3 kg^{-1} FW). The harvest period seems to be a determining factor as nitrate content assimilation in cultivated leafy vegetables is regulated by light intensity [57]. According to these data, none of the study varieties exceeded the

maximum allowable nitrate concentration. Notwithstanding, and depending on starting levels and their evolution, two different behaviors are observed in lettuce: 1) the varieties with a low nitrate transformation rate (to potentially dangerous products), such as L11; 2) the varieties with low initial levels like L5 and L10, which suggests less efficient NO_3^- absorption systems. To support this, the different propensity to accumulate nitrate can be related to: genetic factors [23,24,66]; the variable location of nitrate reductase activity [70]; differential nitrate absorption, transfer and assimilation in plants [23]. It also depends on the quantity available in substrate [24], although this variable is not applicable to our study as all the lettuces were grown under the same field conditions.

Commercial lettuce production also requires adequate potassium levels to provide the high-quality postharvest attributes needed for a longer shelf life [46]. It is known that plant growth and yield are strongly affected by substrate K availability [46,71]. By growing lettuce varieties in the same soil in our study, we were able to compare the K accumulation capacity of our different lettuce varieties. Landrace L11 stood out from the rest, even though it was the worst preserved one during the postharvest period. This confirmed that some varietal genetic differences allow potassium to be taken up or retained more or less easily.

5. CONCLUSSIONS

From the analysis comparing the postharvest evolution in the nutrient composition and visual quality of the five local Valencian lettuce landraces and four commercial varieties, we conclude that:
(1) The initial nutritional quality (T0) depends on lettuce type, especially due to leaf color and structure. In this regard, reddish varieties CL4 and L11 stand out for their high concentration of bioactive compounds.

(2) Postharvest behavior (T3, T6, T9) of lettuces is variety dependent. Among them, landrace L10 highlights as both, its nutraceutical content and visual appearance, are maintained along the storage period.

(3) Regarding other varieties, such as CL4, L2, L5 and L11, visual quality may not correspond to their bioactive properties, as antioxidant compound (anthocyanins, carotenes and phenols) synthesis events occurred during the storage period, likely to react to storage stress conditions.

(4) Based on consumer judgment, we highlight the already commercial varieties CL2 and CL3, followed by landraces L1 and L10, which retained their good appearance (firmness, freshness and color) through storage.

Author Contributions: Conceptualization, Á.C., M.-R.M.-C., and E.M.-I.;

CL1	T0	T3	T6	T9
Firmness description[1]	3,8	3,8	3,8	3,8
Visual quality[1]	8,4	6,9	6,6	6,3
Decay[1]	8,5	6,8	5,5	5,6
Butt discoloration[1]	8,8	7,8	6,4	5,6
Wilting[1]	9,0	7,0	7,4	7,5
Internal part appearance[2]	3,0	3,0	3,0	3,0

CL2	T0	T3	T6	T9
Firmness description[1]	3,5	3,2	3,0	3,0
Visual quality[1]	7,9	6,4	6,4	5,8
Decay[1]	8,7	7,9	7,8	7,0
Butt discoloration[1]	9,0	8,5	7,0	7,0
Wilting[1]	8,9	7,3	7,0	6,5
Internal part appearance[2]	3,0	3,0	3,0	3,0

CL3	T0	T3	T6	T9
Firmness description[1]	3,9	3,3	3,3	3,3
Visual quality[1]	9,0	7,2	7,0	7,0
Decay[1]	8,9	7,5	7,4	6,8
Butt discoloration[1]	9,0	9,0	8,8	7,6
Wilting[1]	9,0	8,2	8,0	8,0
Internal part appearance[2]	3,0	3,0	3,0	2,5

CL4	T0	T3	T6	T9
Firmness description[1]	2,5	2,0	2,1	2,0
Visual quality[1]	7,7	6,5	5,6	5,4
Decay[1]	8,0	7,0	6,8	6,7
Butt discoloration[1]	8,5	8,3	7,8	7,5
Wilting[1]	9,0	6,0	5,3	5,5
Internal part appearance[2]	3,0	3,0	3,0	3,0

	L1	T0	T3	T6	T9	
	Firmness description[1]	2,7	2,5	2,1	2,1	
	Visual quality[1]	8,1	7,6	7,4	6,3	
	Decay[1]	7,7	7,1	6,5	5,9	
-36,3	Butt discoloration[1]	9,0	8,0	7,0	7,0	-22,2
	Wilting[1]	8,9	6,5	6,0	5,8	
	Internal part appearance[2]	3,0	3,0	3,0	3,0	

	L2	T0	T3	T6	T9	
	Firmness description	2,3	2,3	2,3	2,0	
	Visual quality	8,4	7,8	6,8	3,4	
	Decay	8,7	8,3	6,9	3,2	
-21,9	Butt discoloration	8,4	8,3	8,0	6,7	-20,1
	Wilting	9,0	7,8	7,9	7,6	
	Internal part appearance	3,0	2,5	2,5	2,0	

	L5	T0	T3	T6	T9	
	Firmness description[1]	2,8	1,8	1,8	1,8	
	Visual quality[1]	9,0	5,8	5,3	4,7	
	Decay[1]	9,0	7,0	5,7	4,5	
-15,3	Butt discoloration[1]	9,0	8,0	7,8	7,2	-20,0
	Wilting[1]	9,0	7,4	6,8	5,9	
	Internal part appearance[2]	3,0	3,0	3,0	2,2	

	L10	T0	T3	T6	T9	
	Firmness description[1]	4,9	4,9	4,0	4,1	
	Visual quality[1]	8,3	7,5	7,4	7,1	
	Decay[1]	8,4	7,3	6,0	5,6	
-11,8	Butt discoloration[1]	9,0	8,5	8,1	7,1	-20,8
	Wilting[1]	9,0	7,9	6,9	6,6	
	Internal part appearance[2]	3,0	3,0	3,0	3,0	

L11	T0	T3	T6	T9	
Firmness description	1,6	1,4	1,3	1,1	
Visual quality	7,7	5,5	4,0	1,9	
Decay	8,5	4,8	3,3	2,8	
Butt discoloration	9,0	9,0	7,0	4,9	-45,8
Wilting	9,0	5,5	5,1	3,5	
Internal part appearance	3,0	3,0	3,0	3,0	

*** Table S1.** Visual quality (absolute data based on rating scale described in Table 2) of 9 harvested lettuces measured at 3, 6 and 9 days after conservation in chamber conditions. 1External trait, 2Internal trait.

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CHAPTER 08

GENERAL DISCUSSION

A). Pepper and eggplant characterisation: phenotyping and nutritional quality determination of traditional varieties

Phenotyping is an efficient tool for estimating genetic diversity among genotypes because it illustrates existing divergence [1]. In this context, by knowing the importance of eggplant and pepper cultivars worldwide, the complete phenotyping of the selected traditional Valencian varieties for both crops was carried out following the standardised morphological and agronomic descriptor guidelines developed by the International Plant Genetic Resources Council [2–4]. Of the data collected from both trials, it was possible to select those characters of interest preferred by farmers, for harvest practices and by end consumers.

From the two phenotyping assays, a high degree of diversity was established among accessions despite the fact that they all came from the same geographical area (Valencian Community, Spain). This fact is supported by Bianchi et al. [1], Cardoso et al. [5], Baba et al. [6], Moreira et al. [7] and Lahbib et al. [8], who suggest that accessions from the same regions cannot be grouped according to their geographical origin. In fact Muñoz-Falcón et al. [9] suggest that local conditions, in addition to the selection processes followed by farmers, generate differentiation between varieties belonging to the same crop and origin. Moreover, both case crops are considered to be mainly self-pollinated species, although cross-pollination events in contact with air, insects or high temperatures may occur, which can generate a certain degree of diversity between varieties [9,10] and force the genetic isolation of plant populations [11]. The main differential traits between each crop's landraces specifically centre on fruit. This separation of accessions associated with fruit traits has also been described by other authors [2,12,13], which confirms that the morphological variation of the organ for which a crop is selected widens during the domestication process [14].

Regarding the plant vegetative traits of both crops, it was found that larger plants develop bigger leaves (in both length and width terms), together with more flowers and fruit per plant, which translates into higher yields. This is because a larger leaf area better accumulates photosynthates in plants, which are translocated to the production of thicker and wider fruit [15,16]. In addition, although yield is a very complex issue that is influenced by several traits, some authors report a positive correlation between yield and plant size [15–18]. Moreover from the cultivation point of view, narrow genotypes allow more plants per unit area, which implies better utilised cultivation area and higher economic yields.

On pepper, it is known that fruit can determine the most appropriate use. Small pepper fruit with thick pericarps are employed in the processing industry (P-49 could match this profile), while large uniform fruit with good texture are preferred to be sold as fresh produce (all 18 studied varieties could match this grouping) [19]. Moreover, those varieties whose fruit develop elongated pedicels [20] and are moderately persistent (strong enough so that fruit do

not detach from plants even with the effect of metrological events, but fragile enough to favour good detachment when harvested) are desirable traits for cultivation [21]. Some such cases are P-44 and P-50 when green, and P-35, P-36, P-47 and P-50 when red.
For eggplant, the wider and heavier fruit are, the bigger calyx prickles are, which is an undesirable characteristic for harvesting, handling processes and consumer acceptance [22]. A smaller calyx (in relation to the total fruit size) is preferable from a phytosanitary point of view because the humidity generated between the petals that are still adhered between the calyx and fruit promotes fungi propagation [22]. Slightly heavy elongated fruit are preferable in this context. Likewise, pubescence on leaves, no thorns on leaves and erect growth habit are also traits that meet the needs of both growers and end consumers [22]. Some such cases are landraces B4, B9 and B12.

In addition to phenotypical characterisation, the determination of nutritional quality defines a relevant role in crop improvement [23]. The chemical composition of a plant variety, especially that of nutraceutical compounds, can confer the product added value, which coincides with growing consumer concern about the nutritional and nutraceutical values of products and their positive relation to human health [24–26]].

It is important to emphasise that consumers are attaching more importance to the antioxidant properties of foods [27], although taste is also an extremely important factor because consumers will only decide to consume a product again if their organoleptic expectations have been met [28]. Both pepper and eggplant fruit are becoming more relevant on the market by being considered functional foods given their richness in antioxidant compounds [25,27,29,30], together with their unique taste. It is also known that wide variability exists in antioxidant potential among varieties, including the landraces that we selected for our research [31–35].

In particular, both crops are rich in ascorbic acid and polyphenols [15,35,36], which are strong antioxidants [37]. In line with this, we aimed to describe the antioxidant potential of the selected traditional varieties. In pepper, we analysed fruit in two ripening stages (green and red) by quantifying several antioxidant compounds (phenolics, lycopene, carotenes, vitamin C). Of the 31 eggplant varieties, three were selected based on: 1) their differential fruit skin colour (purple, striped and white skin); two agronomic characteristics of interest (data obtained from the phenotyping part). Of our selection, the antioxidant potential (phenols, ascorbic acid, carotenoids, flavonoids; all strong antioxidants) and taste qualities (soluble sugar content and its relation to flavour, which can be key for satisfying end consumers) [38], were determined

As a general rule, ripe pepper fruit contain bigger amounts of phenolic compounds, vitamin C, lycopene and carotenes than green ones. All these results support the notion of the wide variability in landrace behaviour towards the ripening process, a fact that the literature supports in studies performed with commercial pepper cultivars, such as bell and California pepper plants, and with other species like tomato [35,39–41]. It is also important to highlight the intrinsic landrace effect because landraces with more antioxidant capacities upon maturity do not necessarily stand out from the rest in the immature stage. So it is possible to select the most suitable varieties to consume their fruit when unripe (P-36 and P-44) or ripe (P-44 and P-48), and selection is based on total phenolic and vitamin C contents. For lycopene, differences in concentration were more evident after our analysis in immature fruit because the concentration was so low in some landraces that it was not even detected (i.e. P-49 and P-72). In contrast, most

local varieties showed a similar carotene concentration for each fruit ripening stage, where landrace P-44 when green and varieties P-39 and P-50 when red are highlighted.

Phenolic compounds in eggplant have been identified as the main bioactive compounds responsible for its antioxidant effects [42], and it is known that genotypes are very diverse in the proportions of these compounds, as measured by spectrophotometry [43]. The results obtained in our varieties for flavonoids or vitamin C might not compare to the values reported in other crops, or even other aubergine varieties. However, studies like that of Prohens et al. [44] show the existence of significant differences between traditional varieties and commercial hybrids, with the indigenous varieties being richer in ascorbic acid content on average.

In our study, significant differences were observed among these three landraces that stood out: B14 for its antioxidant properties with higher concentrations of phenols, flavonoids, total antioxidant capacity and carotenes than B16 and B17 for its high sugar content; B16 did not stand out for any trait, but its white skin colour could certainly catch customers´ eyes. The significant differences found among accessions suggest not only genotype dependence for the analysed traits, but also wide diversity among the local eggplant landraces, and their crop management and environmental conditions.

The key of both nutraceutical assays lies in the wide variability among landraces, as well as the need to take into account several bioactive compounds to determine the full antioxidant capacity of a given variety without forgetting its agronomic profitability.

B). Nutritional quality among the different lettuce varieties: changes in development stages and under storage conditions

The nutrient content of lettuce is determined by factors, such as genetics, the influence of the environment, the harvest stage of plants and their postharvest treatment (mainly conditioned by temperature, RH and light) [45–50]. It is known that microgreens and baby lettuces can have significantly higher levels of vitamins, minerals and other phytonutrients that benefit health, which is why they are considered functional foods [51]. However, given the standardised consumption of adult lettuce, it is important to deal with varieties with acceptable firmness, texture, colour and nutraceutical qualities, which are attributes that draw consumers to lettuce. In addition, loss of product quality is associated with its deteriorated visual appearance [52].

The main visual indicators of lettuce quality are tissue water status and colour retention. The optimum storage level lies between 95-98% RH [53], which remained throughout the trial, even if certain weight loss was inevitable during storage. Commercial variety CL4 and landraces L5 and L11 were those that wilted the most during storage in the adult phase, even though they were among the most nutritionally rich varieties. This fact could affect consumers' rejection options. On the contrary, CL1, CL3 and L2 would be preferred for the market for being better visually preserved during storage. Colour retention was associated mainly with chlorophyll content preservation [53]. The general tendency was to observe chlorophyll loss during storage with differences in behaviour between

varieties. The chlorophyll content of varieties CL2, CL4 and L5 increased (T3), possibly in response to the stress conditions, although their chlorophyll amount lowered in the end (T6 onwards). Visually, L11 was the variety that underwent the most discoloration during storage. However, we assumed that L11 would be the most susceptible variety to stress, but it was accompanied by a high response potential because, during storage, several antioxidant production events occurred with this landrace, which were possibly activated to cope with stress.

When focusing on nutritional quality, to a great extent the healthy properties that derive from lettuce are due to the large supply of antioxidant compounds, mainly vitamin C and polyphenols, and fibre and mineral content [54]. In this context, studying the nutraceutical properties of the selected lettuce varieties was considered extremely relevant to compare these attributes in different development stages and under postharvest conditions.

Anthocyanins stand out for their strong antioxidant capacity and are known for conferring leaves their red colour [55,56]. This is consistent with the higher Ant content of red variety L11, at least from the baby green stage onwards. In microgreens, colour did not seem to be a clear indicator of pigment content (green variety L1 is among the richest varieties in Ant). Moreover, several authors like Llorach et al. [54], Baslam et al. [49] and Kim et al. [46] claim that red pigmentation is indicative of Ant, together with total Phe content. As Ant are a type of polyphenol, it makes sense that the observed trend in the total phenol concentration coincides with the trend noted in Ant content to a great extent. This falls in line with the positive correlations found between both parameters in our trial, together with the total antioxidant capacity in the baby and adult lettuces. However, this correlation was not observed in the microgreen stage, perhaps even if Ant production was high at the time. Furthermore, Ant content rose during the postharvest period, which suggests that it is a highly relevant pigment during lettuce conservation. However, the total Phe contents of the microgreen and baby lettuces were 79% higher compared to the adult stage, while the Ant contents of all the young seedlings were only 11% higher than those found in adult plants. Hence the production of non-anthocyanidic phenols may occur or start before the production of Ant themselves, or even be much more abundant.

According to the results, the highest vitamin C content was observed in microgreens and baby greens compared to adult lettuces. This event was also noted by Xiao et al. [57] in many different plant species. This indicates that the biosynthesis of the compound is activated early in development and then its rate gradually normalises. Despite the direct relation among Ant, phenols and vitamin C at harvest for being the main antioxidants in lettuce, this correlation was broken during the storage period. This was because, unlike the production peaks observed during the conservation of Ant and phenols, vitamin C content proportionally lowered with time in all the studied cases. This supports the notion that the postharvest decay of fruit and vegetables is due mainly to the continuous degradation of their own nutrients [58,59]. However, the sudden production of other antioxidants indicates plants' ability to respond to the conditions imposed during the conservation period. In fact the lipid peroxidation rate rose due to the stress situations caused by storing lettuce, but was then counterbalanced by the rapid production of mainly Ant. All this would imply that vitamin C is not actively involved in this immediate response to stress. Undoubtedly the antioxidant capacity of vitamin C cannot be denied, and varieties CL4 and L11 are highlighted for their high AsA content in the three development stages. However, landrace L10 would be preferred for to its vitamin C preservation ability under storage conditions.

As far as the concentration of carotenes is concerned, we see that both varieties and the conservation process visibly increase the content of this pigment type, slthough the time when these production events occur seems to be variety-dependent. Therefore, and in relation to what is proposed above, carotenes would be part of the direct response to postharvest stress. Likewise, the positive correlations detected between carotenes and other compounds, such as phenols (T0, T3), vitamin C (T6) and Ant (T9), highlight their antioxidant properties. The antioxidant properties of carotenoids have also been previously mentioned by [60,61]. When focusing on lettuce development, varieties reached the highest carotenoid content in the baby green stage. This might imply that carotenoid biosynthesis is accentuated or enhanced during this growth period to cover, together with chlorophylls, light harvest complex and photo-protection duties by neutralising ROS [62] and, thus, enhancing plant development.

Finally, mineral content in plants depends on the substratre composition because it is the main resource from which the plant obtains these kind of elements. The final mineral concentration depends basically on lettuce type and its capacity to capture or retain a certain concentration of the main minerals, such as potassium, calcium or iron. Potassium is required for normal plant growth, photosynthesis, transpiration, development and yield [63–66], while calcium and iron enhance photosynthetic activity and chlorophyll synthesis [64,67]. In this context, the leaves of reddish varieties CL4 and L11 presented the highest calcium and iron accumulation in the adult stage, while CL2, CL3, L1 and L3 stood out for their high potassium concentration and CL3 for its high iron content. They are highlighted for encompassing beneficial mineral uptake properties, which are relevant factors for variety selection. Although nitrate is known for being crucial for plant growth and development [68], nitrate reaction products and their accumulation in leaves are potentially dangerous for health [69,70]. Hence those varieties with low nitrate accumulation tendency in leaves would be of more nutritional interest, for which landraces L2, L5 and L10 are preferable.

By jointly comparing all the data, it is possible to choose lettuce varieties with a higher nutritional value according to development stage and resistance to postharvest practices. The potential of local lettuce varieties is also emphasised for being a relevant biodiversity source.

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CHAPTER 09

FINAL CONCLUSIONS

The knowledge acquired in this doctoral thesis can be summarised in the following key points:

1. This study highlights the significant existence, but scarcely exploited variability, of landraces that belong to three important crops (pepper, aubergines, lettuce), which make them key elements for the conservation and promotion of horticultural biodiversity

2. Phenotypical description complements nutritional knowledge to analyse variability in crop diversity studies, and to identify valuable accessions for breeding programmes and agronomic management, to obtain easily manageable and harvestable varieties, develop efficient conservation strategies and produce detailed agricultural catalogues thar facilitate optimal variety selection according to each situation

3. Nutraceutical properties can be modified depending on agronomic management and environmental conditions. In this context, as the nutritional profile is helpful for promoting the commercialisation and consumption of local varieties, the optimal variety type and harvesting period can be chosen to achieve the desired crop quality

4. The results of the present study show that:

   4.1. For pepper traits, in addition to establishing the optimal morphological characteristics for harvesting activities (including erect growth habit, dense branching, large leaves, and fruit uniformity and low persistence), it is feasible to determine the possible destination of pepper use based on the size and thickness of fruit walls. In this context, the most appropriate varieties to be marketed fresh can be P-37, P-41 and P-72, while P-49 would be an optimal candidate for industry processes for its compact size and fruit wall robustness. In nutritional quality terms, it is important to highlight the differences between green and red fruit: mature fruit are related to high vitamin C and carotenoid contents, while green ones are associated with high polyphenol contents. Variety P-44 is highlighted for its high nutraceutical properties in both green and red stages

   4.2. Of the aubergine landraces, varieties B4, B12 and B19 are highlighted because their traits were the preferred characteristics for handling jobs (erect growth habit, low branch density, lack of hair on leaves, no prickles on the calyx, and the development of elongated, and not excessively heavy, fruit). When focusing on nutraceutical properties, purple landrace B14 is richer in antioxidant compounds. However, for other varieties likes B16, commercialisation can be promoted for their distinctive and attractive white skin colour

   4.3. The nutrient content of lettuce depends on lettuce type, leaf colour and development stage. The most recommendable stages to harvest lettuce plants are the micro- and baby green stages when antioxidants are much more concentrated. In the adult stage, this bioactive profile depends mainly on the head structure and pigmentation rate (Ant content, for which reddish varieties CL4 and L11 stand out). However, the postharvest behaviour of lettuces is variety-dependent. Variety L10 is clearly highlighted because both its nutraceutical content and visual appearance remained during storage