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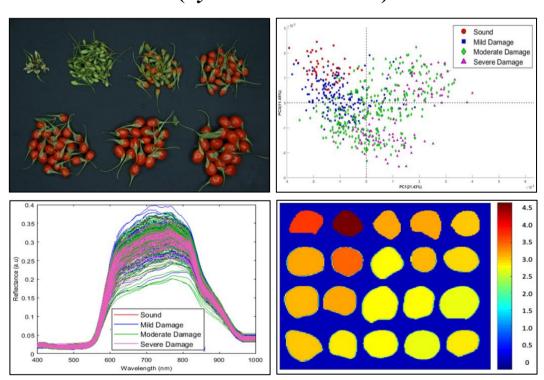
Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria

Doctoral Thesis in

Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region

- XXXIII cycle -

Increasing Knowledge on Postharvest Handling of Goji Berries (*Lycium barbarum* L.)



Candidate:

Danial Fatchurrahman

Tutor:

Prof. Maria Luisa Amodio



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Table of Content

EXT	TENDED ABSTRACT	9
1.	. Goji importance and diffusion	14
2.	. Quality of Goji Berry	15
	2.1. Color	15
	2.2. Size	15
	2.3. Nutrient Components	16
	2.4. Flavor and Taste	16
	2.5. Main causes of deteroriation	17
3.	. Factors Influencing the Quality and Storability of Goji Berry	18
	3.1. Variety	18
	3.2. Maturity Stage	19
	3.3. Harvest Time	19
4.	Postharvest Strategies to Prolong Shelf-life	20
	4.1. Environmental condition	20
	4.2. Atmosphere composition	21
	4.3. Chemical treatments	24
	4.4. Plant extract as alternative preservatives	26
5.	. Assessing Quality of Small Fruit	27
	5.1. Digital imaging	28
	5.2. Spectroscopy	29
	5.3. Hyperspectral imaging	32
R	eference	36
Gen	neral objectives	42
	aracterization and Postharvest Behavior of Goji Berry (Lycium	
bar	barum L.) During Ripening	44

ABSTRACT	45
1. Introduction	46
2. Material and methods	47
2.1. Sample preparation	47
2.2. Physical attributes	49
2.3. Chemical	49
2.4. Respiration rate and ethylene production	51
Result and discussion	52
3.1. Characterization of the 6 classes at harvest	52
3.2. Fruit quality changes after storage	61
4. Conclusion	65
References	67
Postharvest Physiology and Quality of Goji Berry (Lycium barbarum L.)	Fruit
Stored at Low Temperatures	70
ABSTRACT	71
1. Introduction	72
2. Materials and methods	73
2.1 Sample preparation	73
2.2 Physical quality attributes	74
2.3 Sensorial analysis	74
2.4 Electrolytic leakage	75
2.5 Physiological and metabolic attributes	75
2.6 Maturity index	76
2.7 Chemical composition	76
3. Statistical analysis	79
4. Result and discussion	79

5. Conclusion.	91
Reference.	92
Effect of Controlled Atmosphere with High Carbon Dioxide on Quality of	
Goji Berry Fruits (Lycium barbarum L.)	103
ABSTRACT	104
1. Introduction	104
2. Material and method	106
2.1. Sample Preparation	106
2.2. Sensorial analysis	107
2.3. Soluble solid content, titratable acidity and pH	107
2.4. Determination of vitamin C	107
2.5. Total polyphenol and antioxidant activity	108
2.6. Statistical analysis	108
3. Results and discussions	108
4. Conclusion.	118
Reference	118
Comparison Performance of Visible-NIR and Near-Infrared Hyperspectral	
Imaging for Prediction of Nutritional Quality of Goji Berry	129
ABSTRACT	130
1.Introduction	130
2.Materials and methods	132
2.1. Sample preparation and spectral acquisition	132
2.2. Hyperspectral image acquisition	133
2.3. Chemical analysisis and Partial Least Square Regresion (PLSR)	133
3. Results and discussion	137
3.1. Spectral and spatial profile	137

3.2. Comparison of prediction model between spectra range VIS-NIR and NIR	
4. Conclusion	149
Reference	150
Classification of Common Defective Goji Berry (Lycium barbarum L.) Using	
3.2. Comparison of prediction model between spectra range VIS-NIR and NIR	160
ABSTRACT	161
1. Introduction	161
2. Materials and methods	163
2.1. Fruit samples	163
2.2. Quality assessment	164
2.3. Hyperspectral image acquisition and spectra extraction	165
2.4. Chemometric	166
3. Results	167
4. Discussion	174
5. Conclusion	178
Reference	179
GENERAL CONCLUSIONS	180

EXTENDED ABSTRACT

Goji berry (*Lycium barbarum* L.) is a fruit of solanaceous family that is widely found in arid and semi-arid regions of northwestern China, southeastern Europe and the Mediterranean. China represents the biggest producer worldwide representing its 85,000 ha of cultivated field and 98,000 t of fruits produced per year. Goji berries, also known as wolfberries, traditionally utilized in Chinese medicine, have become increasingly popular in the western world because of their nutritional properties. Generally, goji berries are cooked and processed as tea, soups, or served with meat and vegetables. They are also utilized for juice, tincture, and wine production. The fruit is also consumed in dried form or processed in powdered form for medicinal purposes. Given their high perishability, high water content, and susceptibility to damage and rot, fresh goji berries are generally available in areas where they are cultivated. This is also due to the lack of information on goji berry postharvest behavior and storage recommendations.

From a pomology point of view, goji berry's first fruition is typically observed in 3-year-old plants. The mature fruit is 1 to 2 cm long, with an ellipsoid shape and a bright orange-red color similar to mature mini-tomato, and contains 20–40 tiny seeds per fruit. It is sweet and has a tangy aroma. It is reported that the green stage is the most appropriate for extracting organic acids for pharmacological purposes. The red stage is processed into dried fruit for direct consumption, benefiting from sugars and L-ascorbic acid content. There is no indication of its use as a fresh fruit. However, there is no clear information regarding the characterization of fruit maturation stages, and the classification in terms of climacteric or non-climacteric fruit has not been explored. Therefore, we identified several maturity indexes from different stages of maturity, including physical properties such as length, width, weight, color (hue), firmness, soluble solid content (SSC), pH, and titratable acidity (TA). Fruit dimensions increased from class 1 to 6 starting from 8.08 mm length, 3.95 mm width, and 0.07 g of weight, to 16.26 mm, 13.15 mm, and 1.29 g, respectively. Soluble

solids increased from 2.68 % to 23.5 %. Goji berries showed climacteric behavior indicated by a climacteric peak during the early stages of development. This included changes to TA and SSC, which from class 4 to 6 stabilized around the maximum value of 23 % after eight days of storage at room temperature. Their high nutritional value was confirmed by the content of vitamin C, which is comparable to that of citrus fruit. It reached the maximum value of 0.52 g/kg at full ripening, whereas the phenolic content decreased during ripening to values of 2.15 g/kg. This contributed considerably to the high antioxidant content of the berries.

Temperature and relative humidity are the first storage conditions to be accurately controlled to preserve quality and allow maximum shelf-life. Studies on goji berry have only considered low (0 and 2 °C mostly) or high (10 or 20 °C) temperatures. Several studies reported the effect of temperature on postharvest quality of fresh goji berry fruit during storage at different temperatures (-2, 0, 10, and 20 °C) and concluded that 0 °C was the optimum temperature to maintain the berry phytochemical and sensory qualities. However, these results are not reliable because of the large temperature gap among the tested temperatures and did not include refrigeration temperature of 5 °C, which is used in the cold chain of fresh or minimally processed products for transport and sale. As goji berries belong to the Solanaceae family, they are plausibly chilling-sensitive, such as tomato, bell pepper, and eggplant. However, the quality of fruit stored at 0 °C may show a slower degradation compared to that of fruit stored at 10 or 20 °C. Therefore, one experiment was aimed to test the effects of low storage temperatures between 0 and 7 °C to identify the best storage temperature for maintaining goji berry quality and shelf-life, with particular attention to the occurrence of chilling injury symptoms. Fruit stored at 0 °C showed the lowest respiration rate and ethylene production (5.82 mL CO₂ kg⁻¹h⁻¹ and 0.667 μL C₂H₄ kg⁻¹h⁻¹, respectively); however, at this temperature, the highest incidence and severity of shriveling and dark spots was observed reaching 59 and 15.42 %after 12 days of storage. In contrast, 5 °C was found to be the best storage temperature for goji berry fruit; the fruit appeared fresh and healthy and had the highest scores during sensory analysis with a general acceptable impression, with the lowest damage attributable to chilling injury; 27.14 % fruit presenting shriveling; 6.17 % black spot damage. Storage of goji berries at 7 °C resulted in the lowest marketability and the highest incidence of decay due to accelerated senescence processes. Significant differences were also found in the phytochemical attributes, vitamin C content, SSC, TA, SSC/TA ratio, total polyphenol content, DPPH, and anthocyanin content.

Another important technology to prolong the shelf-life of fresh fruit is controlled atmosphere (CA) storage. Increasing CO₂ concentration respect to air levels, depending on crop tolerance, may results in shelf-life extension due to its fungistatic effect and to the inhibition of ethylene synthesis and action. Fruit were stored in air enriched with 5 %, 10 %, 20 % CO₂, and 0 % for the control in air, for 22 days at 5 °C. Goji fruit stored under CO₂ concentration of 20 % showed the highest quality allowing a reasonable shelf life up to 20 days. Fruits stored at 5 % and 10 % and in air showed higher susceptibility to decay represented as firmness losses and severity of mold infection. Regarding chemical composition main differences regarded antioxidant activity, which increased for the treatment at 5 % and 10 %.

Optical fruit sorting and selection is a very critical step of the handling of fruit. The demand for a rapid, and non-destructive tool for the selection of defective fruit is increasing. Moreover, internal quality and composition is a very valuable information which can also be obtained thanks to the potentiality of new optical methods. Hyperspectral Spectroscopy Imaging (HIS) is one of the tools to obtain information about external and internal quality of fruit, with an added value for small fruit, very sensitive to manual handling and manipulation. HIS was used to discriminate defective from sound fruit. As for common damages, the defects were identified as mild (i.e. visual damage, softening, bruise), moderate (i.e. pitting, initial mold), and severe damages (i.e. severe mold). Hyperspectral imaging (HIS) system in the Vis-NIR ranges was used for the defect detection using visual appearance analysis, as reference measurement. The outcomes of this study indicate the

promising potential for visible-NIR to provide non-invasive, rapid, and reliable early detection of common disorders in goji berry fruits.

As for the prediction on the internal composition in term of Vitamin C, total antioxidant, phenols, anthocyanin, soluble solids content (SSC), and total acidity (TA), Hyperspectral Imaging (HIS) in the Visible- Near Infrared (VIS-NIR) (400–1000 nm) and in the Near Infrared (NIR) (900–1700 nm) was applied. For vitamin C and AA, partial least square regression (PLSR) combined with different data pretreatments and wavelength selection resulted in a satisfactory prediction in the NIR region obtaining the R²pred value of 0.91. As for phenols, SSC, and TA, a better performance was obtained in the VIS-NIR region yielding the R²pred values of 0.62, 0.94, and 0.84, respectively.

PART I: INTRODUCTION

1. Goji Importance and Diffusion

Goji berry fruit belongs to the genus *Lycium* in the family Solanaceous. It is widely found in the altitude range from 700 to 2700 m and has a broad harvesting period from June to the end of October. The plant is tolerant to excessive hot and extreme cold, drought, and other environmental stress conditions. *Lycium barbarum* L is the most widely grown species, and the color is ranging from orange to dark red. Dried goji berries of the species *Lycium barbarum* L have been used widely in china as medicinal purpose and for snacks (Jin et al., 2013). Goji berries are now widely distributed in northwestern China, the Himalayas, Mongolia (Potterat, 2010), southeastern Europe, and the Mediterranean region (Redgwell et al., 2011).

Due to their rich nutrients goji berry fruit are considered as the latest super fruit and globally accepted as a functional food, including high contents of polysaccharides, flavonoids, carotenoids, amino acids (Aa), mineral elements, vitamins, and betaine. The medicinal properties of goji berries are very effective as blood glucose control and serum lipids; nourishing eyes, kidneys, and liver; improving immunity; and enhancing hemopoiesis, and it has pivotal roles in anti-radiation, anti-aging, anti-cancer, and anti-fatigue (Luo et al., 2004).

In recent years, fresh fruit consumption of goji has become more popular due to consumer's increasing demand for healthy, nutritious, and natural foods (Kafkaletou et al., 2018; Potterat, 2010). Goji berry fruit are usually distributed and consumed as a dried product. However, this is often accompanied by the loss of functional components such as carotenoids, vitamins. amino acids, proteins, and fatty acids during the drying process (Hu et al., 2011). With the development and improvement of postharvest strategies, fresh goji berries have been packaged in boxes for the domestic and international fruit markets. Fresh goji berries harvested with or without their stems have been available to fresh fruit markets in Asia, central and northern Europe, and North America.

Nevertheless, fresh goji berry fruit are still a little-known fruit species due to their

high perishable nature that greatly limits their circulation and marketing value. The shelf-life is only for 1–2 days at room temperature and 5–7 days in a kitchen refrigerator. Harvested goji fruits face several problems, such as susceptibility to pathogens, unsatisfactory flavor, and damages. The challenge is to find an optimum temperature and effective approaches to reduce the postharvest losses, improving quality, and prolonging the shelf life of fresh goji berries (Jatoi et al., 2018).

2. Quality of Fresh Goji Berry

2.1. Color

Goji, like tomatoes, also belongs to the Solanaceae family and have peaks of respiration rate and ethylene production rate and suggested to be a climacteric fruit (Zhou et al., 2020). The fruit contains a lot of carotenoids, which are lipid-soluble pigments that range in hue from yellow to red. Because of the presence of carotenoids, red cultivars are the most demanded after of all the goji color variants available. The predominant form of carotenoid found in goji fruits is zeaxanthin dipalmitate, which accounts for more than 80% of the total carotenoids (Inbaraj et al., 2008). According to Mi et al. (2018), total carotenoid content was strongly associated favorably with redness (a) but adversely with hue angle (h). Brightness (L) and hue angle (h) were favorably connected with zeaxanthin content, while redness (a) and saturation (c) were positively correlated with zeaxanthin dipalmitate content.

2.2. Size

Appearance has become is one of the most important sensory factors for market acceptance. Thus, the appearance of fresh goji fruit has become an essensial indicator of whether goji varieties are suitable for fresh fruit consumption. After comparing the size and length width ratio of different varieties of goji fruits, Huang et al. (2013) found that large goji berry fruit with the horizontal diameter of fresh

goji over 26 mm, the ratio of vertical to transverse diameter with 0.4:1, with bright color were more popular and accepted by consumers. The pattern of pigment accumulation in n goji berries was similar to that of tomato. Moreover, grafting tomatoes on goji plant (*L. chinense* Mill.) produced smaller tomato fruit with lower fresh weight and improved fruit quality, including total soluble solids (TSS), titratable acidity (TA), ratio of TSS/TA, and vitamin C (Vc) (Huang et al., 2015).

2.3. Nutrient Components

A study conducted by Shu et al. (2017) analyzing the nutrient compositions of 22 different varieties and reported that goji fruits were rich in polysaccharides (4.12–15.49 mg/g) and betaines (1.23–7.36 mg/g); the predominant flavonoids were identified as rutin (19.35–131.90 μ g/g). The total carotenoid contents ranged from 1.22 to 283.62 μ g/g, and the main components of carotenoids were zeaxanthin, lutein, β -cryptoxanthin, β -carotene, and neoxanthin. The cluster analysis of 22 different varieties demonstrated that goji berry varieties could be divided into four types according to the comparison of nutritional components. Type 1 contained significantly higher contents of rutin, zeaxanthin, lutein, and neoxanthin. Type 2 contained the highest amount of polysaccharides and β -cryptoxanthin, but the lowest amount of betaine, rutin, β -carotene, and neoxanthin. Type 3 contained the highest contents of betaine and β -carotene, while type 4 contained the lowest amount of polysaccharides, zeaxanthin, lutein, and β -cryptoxanthin (Shu et al., 2017).

2.4. Flavor and Taste

Flavor substances and functional ingredients are the important factors affecting the quality of fresh fruit. Eight typical indices, including 2 yield-related indices (vertical diameter and horizontal diameter), 4 flavor substances (fructose, glucose, oxalic acid, tartaric acid), and 2 functional ingredients (flavonoids and polysaccharides) were screened from 24 quality indices of fresh goji fruits by correlation and factorial

analysis. The analysis results suggested that the quality of fresh goji fruits with different colors could be evaluated by the eight previous indices. The comprehensive evaluation values of quality showed a significant difference among goji fruits of different colors, with the following order: red fruit > purple fruit > yellow fruit > dark red fruit > black fruit (Zhao et al., 2017). The monosaccharides in goji berries contained glucose, fructose, xylose, rhamnose, erythritose, ribose, arabinose, fucose, mannose, galactose, and sorbose. The oligosaccharides mainly consisted of maltose and sucrose. The total sugars were composed of glucose, fructose, sucrose, and oligosaccharides. These were the sources of the sweet taste of goji fruits. Three kinds of key enzymes, sucrose phosphate synthase, sucrose synthase, and invertase were key enzymes involved in the transformation of sucrose in goji berries (Cao et al., 2017). Additionally, organic acids play important roles in the nutritional quality and organoleptic characteristics of fruits, such as taste, flavor and texture (Petkovsek et al., 2007). Citric acid was the main organic acid present in organic and conventional goji berry samples (0.90 and 1.14 g/100 g, respectively), followed by oxalic, tartaric, fumaric and lower concentrations, malic and quinic acids. The content of all organic acids identified, except for quinic and fumaric, showed a significant difference between organic and conventional fruits. Different organic acid contents in goji berry samples were determined in the literature. Citric and malic acids were the main organic acids present in goji berry, identified by Petkovsek et al. (2012), while for Donno et al. (2015) the main organic acid was the quinic. These variations can be explained by the influence of factors such as cultivar, stage of maturation, fertilization, irrigation and soil composition (Feltrin, 2002).

2.5. Main Causes of Deterioration

Goji berries are known as perishable fruit. Given also their high-water content, the fruit is susceptible to damage and rot. The main causes of deterioration of goji berry is fungal pathogens that damage the fruit during preharvest and postharvest phases.

One of the most common postharvest diseases of goji is anthracnose, also known as goji black fruit disease, caused by the fungal pathogen Colletotrichum gloeosporioides Penz (Zhang et al., 2005), and gummosis caused by Cephalosporium acremonium (Yanting & Jianli, 2014). Sun et al. (2013) compared the inhibitory effects of nine fungicides on C. gloeosporioides and reported that the effects of erythromycin and pyrimidate were superior to that of chlorothalonil. Additionally, Verticillium dahliae is a vascular pathogen with a wide host range causing root rot and wilting in the fruit, which have been reported recently in Turkey (Inderbitzin et al., 2011; Özer & Bayraktar, 2016). Furthermore, just like tomatoes, calcium deficiency related abiotic disorder resulting in a water-soaked spot on the end of fruit, where a careful irrigation to minimize extreme fluctuations in soil moisture (particularly during bloom and fruit sizing) can solve this problem. Particularly during storage, temperature gives a significant effect on the development of decay in goji berry fruits. Jatoi et al. (2017) reported that goji berry fruits possessed a healthy appearance if stored under 0 °C, while fruits stored at 10 °C showed the highest deterioration in the form of fruit softening, shriveling, and cracking. Whereas, the fruit stored at -2 °C showed a shriveling disorders while at room temperature of 20 °C, the fruit showed major deterioration of fungal infection.

3. Factors Influencing the Quality and Storability of Goji Berry

3.1. Variety

The goji variety suitable for fresh-eating should be bright, juicy, and sweet, with the qualities of reasonable size, diversity of colors, thicker pericarp with good intensity and tenacity, good taste with appropriate ratio of the contents of TSS/TA, moderate crispness and firmness, small amount of seeds, thick flesh, and reasonable shelf life. (Huang et al., 2013). The quality, composition of nutrients, and storability among different varieties with different developmental stages could be vastly variable. The contents of total sugar in "Ningnongqi No.4" and "Ningnongqi No.5" were

significantly higher than that in "Ningqi No.1" and "Ningxiahuangguo" in mature fruits (Wang et al., 2019 a, b). Zao et al. (2018) compared the storability of six different goji varieties and reported that "14-401" had the best storability, followed by "16-23-7-8," while "Ningqi No.7" had the worst storability.

3.2. Maturity Stage

Wang et al. (2019b) compared the sugar contents in goji fruits of four varieties with three developmental stages and reported that the contents of total sugar, polysaccharides, fructose, glucose, and sucrose in goji fruits increased gradually during the fruit development and maturation. The contents of polysaccharides reached the peak value at the mature stage in "Ningnongqi No.4" and "Ningxiahuangguo," while they reached the peak value at the break stage in "Ningnongqi No.5" and "Ningqi No.1". Significant differences were observed in contents of total soluble solids, titratable acids, and Vitamin C; respiration rates; production rates of C₂H₄; and activities of peroxidase (POD) and pectinase in goji fruits, with different developmental stages (yellow, break, and red), stored under ambient and low temperatures (4 °C). Low temperature storage inhibited the increase of respiration rate and pectinase activity and delayed decreases of TA contents and POD activity and the loss of Vitamin C (Bu et al., 2019).

3.3. Harvest Time

To be harvested in different seasons affects the nutritional components in goji fruits. The summer fruits contained higher amounts of Vitamin C, total acid and water content, while the autumn fruits contained higher amounts of total sugar, total soluble solids, protein, and lipids (Han et al., 2016). Zhao et al. (2015) reported that the vertical and horizontal diameters of goji fruits harvested in summer (July) in Xinjiang province were larger, with higher contents of Vitamin C and lower

accumulation of copper and cadmium than those harvested in autumn (October); thus, goji fruits harvested in summer were more suitable for fresh consumption, and that harvested in autumn was more suitable for making dried goji berries.

4. Postharvest Strategies to Prolong Shelf-Life

4.1. Environmental Conditions

4.1.1. Temperature

Temperature is the most important factor limiting the shelf life of fresh commodities. Low temperature storage can slow down the respiration rate, reduce metabolism, inhibit the reproduction of microorganisms, delay the speed of infection, allowing longer storability. Thus, low-temperature storage has been widely applied for horticultural products, provided that no chilling sensitivity is observed. Sensitivity to low temperature varies with the species being the tropical and sub-tropical one, genetically more prone to develop chilling symptoms. As goji berries belong to the Solanaceae family, they are plausibly chilling-sensitive, such as tomato, bell pepper, and eggplant (Fatchurrahman et al., 2015; Tsouvaltzis et al., 2020). Thus, further study to understand chilling sensitivity of goji berry is needed to understand if goji fruit can benefit the low temperature storage. Zhao et al. (2010) showed that the respiration intensity, relative permeability of cell membrane, and water loss rate of fresh goji fruits stored at 1°C and 8 °C were significantly lower than those stored at 20 °C, and the contents of Vitamin C and TSS were significantly higher than those stored at 20 °C. Another study on goji stored under different storage temperatures (-2, 0, 10 and 20 °C), reported that the fruits stored at 0 °C and -2 °C appeared with lowest weight losses (13.08 and 14.95%) and significantly of better quality compared to fruit stored at 10 °C (18.29% weight loss) for 12 days of storage. Fruits stored at 20 °C deteriorated within a day due to fungal decay, wherease some storage disorders like cracking, peel disorder, shriveling were observed in the fruits stored under -2 and 10 °C. There were significant differences in the phytochemical attributes like SSC, TA, SSC/TA ratio, total polyphenols, DPPH, ABTS and βcarotene while the amount of anthocyanins and CIE color variables were found non-significant. In addition, the fruits stored under 0°C appeared fresh and healthy and hence received highest scores during sensory analysis (Jatoi et al., 2018). While providing relevant information, in these studies there is a large gap among the tested temperatures, not including the refrigeration temperature of 5 °C, which is used in the cold chain of fresh or minimally processed products for transport and sale.

4.2. Atmosphere Composition

Controlled atmosphere (CA) storage has been demonstrated to reduce the respiration rate of fruit and vegetables, provided that the gas level is optimized for the commodity. For some crops in certain conditions, high CO₂ or low O₂ can have either no effect or an increasing effect on respiration rates. The reasons for this variability are many. Generally, there is a specie-specific oxygen threshold below which respiration shifts to anaerobic, increasing CO2 production. Generally, the occurrence of anaerobic metabolism is accelerated at higher temperature. High levels of CO₂ can actually injure the crop, which again could affect its rate of respiration. These effects on respiration rate could also affect the eating quality of fruit and vegetables. Generally, crops stored in controlled atmospheres have a longer storage life because the rate of the metabolic processes is slower. Particularly with climacteric fruit this would slow down ripening and deterioration, so that when they have been stored for protracted periods, they may well be less ripe than fruits stored in air. The actual effects that varying the levels of O₂ and CO₂ in the atmosphere have on crops vary with such factors as the:

- species of crop
- cultivar of crop
- concentration of gases in store
- crop temperature
- stage of maturity of crop at harvest

- degree of ripeness of climacteric fruit
- growing conditions before harvest
- presence of ethylene in store
- pre-storage treatments.

There are also interactive effects of the two gases, so that the effect of CO_2 and O_2 in extending the storage life of a crop may be increased when they are combined. The effects of O_2 on postharvest responses of fruit, vegetables and flowers were reviewed and summarized by Thompson (1996) as follows:

- reduced respiration rate
- reduced substrate oxidation
- delayed ripening of climacteric fruit
- prolonged storage life
- delayed breakdown of chlorophyll
- reduced rate of production of ethylene
- changed fatty acid synthesis
- reduced degradation rate of soluble pectins

Kafkaletou et al. (2017) investigated the effects of short-term (2 days) treatments of high CO₂ and low O₂ concentrations on the storability of fresh goji berries stored at 1° C for 21 days. The treatments with 5% O₂ + 15% CO₂% and 20% O₂ + 20% CO₂ achieved the best effects after 14 days of storage. The treatments did not affect the fruit color, decreased the weight loss, and prevented fungal decay.

In comparison to CA, Modified atmosphere (MA) storage is a low-cost and easily employable technique, which modify the atmosphere within a package, also as result of product respiration. The appropriate packing material is selected so that it can maintain desired CO₂ and O₂ concentration inside the package. Ge et al. (2008) investigated the effects of three types of polyethylene (PE) films with different thicknesses on the quality of goji fruits stored at 4°C and suggested that 0.05-mm-thickness PE film exhibited the best effects in maintaining the contents of Vitamin

C, and soluble sugars. In another study, a 0.03-mm-thick PE film reduced the rates of weight loss and maintained good appearance and taste. The low temperature combined with PE film packaging reduced the rotting rate efficiently, maintained a better sensory quality, delayed the decline of TSS and TA, and effectively extended shelf life, maintaining the quality of the fresh goji berry (Li et al., 2011).

Finally, another method to regulate gas changes, between the inner side and outer side of the fruit is the use of coating material, which form a semi-permeable membrane on the surface. This composition is essentially affected by two processes: the first is respiration, which reduces the internal oxygen partial pressure and increase internal carbon dioxide partial pressure and secondly, diffusion which has the opposite effects (Banks et al., 1993). The positive effects of postharvest coating with chitosan (Chen et al., 2011; Li et al., 2011a), lecithin (Jatoi et al., 2017), zein (Wang and Yang, 2018), and composite coating with lotus leaf extract on the storage of goji berries have been documented. Chitosan coating helped to preserve the quality of fresh goji fruit. Coating with 1.25% chitosan decreased the rates of the fruit decay and weight loss and maintained the content of Vitamin C and TSS in goji fruits stored at room temperature (Li et al., 2009). Coating with 1.5% chitosan could reduce the rot rates of goji fruits stored at 4 °C and delay the decrease of Vitamin C content and accumulation of malondialdehyde (MDA) and the increase of POD activity (Li et al., 2011a). It was reported that goji berries exposed to 5.0 g/L chitosan and packaged with PE bags had a shelf life of approximately 30 days when stored at 0 °C (Chen et al., 2011). Lecithin is a mixture of oleic, stearic, and palmitic acid esters with glycerophosphoric acid and choline. It is recognized as a non-hazardous compound and does not have any specific limitation on its use in food. Application of low dose of lecithin at 1 g/L significantly reduced the decay rate and showed significant improvements in total weight loss, TSS/ TA ratio, chlorophyll (chl) content, and sensory attributes for the 8 days of storage (Jatoi et al., 2017). Zein has film-forming properties when it is dissolved in 80% EtOH solution. Zein coating decreased the rot rate and accumulation of MDA and maintained lower POD activity and higher Vit C content (Wang and Yang, 2018). Composite coating with lotus leaf extract effectively decreased the weight loss, decay rate, and MDA content and maintained the contents of Vit C, TA, and TSS and activities of SOD, CAT, and POD, thus extending the shelf life of goji berries for about 4 days (Fan et al., 2019).

4.3. Chemical Treatments

Among chemical alternative used for postharvest applications, nitric oxide (NO) is a stable free radical and bioactive molecule which plays a pivotal role in the plant system as a signaling molecule. NO is widely known as a plant growth regulator in regulating plant maturation and senescence. Treatments with 0.5 mmol/L sodium nitroprusside, a donor of NO, could effectively reduce weight loss and disease index, maintain TSS content, inhibit ethylene production and respiration rate, and enhance activities of SOD, CAT, and POD in fruits (Feng and Zhang, 2016).

Salicylic Acid (SA) is a plant hormone involving in many physiological, growth, and developmental processes such as photosynthesis, transpiration, ion uptake, and transport. The application of SA helped in inhibiting the growth of fungal pathogens and reducing the postharvest decay of goji fruits. Fungal pathogens were isolated from decayed goji berry fruit and identified according to morphology and internal transcribed spacer (ITS) sequence information. Different concentrations of SA were used and the inhibitory effects of SA on isolated pathogen strains was investigated, namely *Alternaria gaisen*, *Alternaria alternate*, *Fusarum* spp and *Cladosporium* spp., respectively. The result showed that SA treatment inhibited the growth of all fungal pathogens, and that the inhibitory effects increased along with the concentration of SA. Additionally, SA treatment did not give effect on the soluble solid content of the fruit (Wang et al., 2018).

Giberellic Acid (GA) treatment could evidently delay the senescence of fruit peduncle, maintain its freshness, reduce the probability of wound formation in the fruit abscission part and the infection of pathogens, thus extending the storage time and maintaining the quality of goji fruits (Yuan et al., 2011).

1-methylcyclopropene is a safe and efficient ethylene receptor inhibitor, which can maintain quality of various horticultural products by inhibiting in this way, ethylene action. Wang et al. (2012) compared effects of 1-MCP and modified atmosphere packaging (MAP) on fresh goji fruits stored at 2 ± 1 °C. It was concluded that 1-MCP treatment could alleviate the sour taste of goji fruits at the end of storage but had no significant effects on peel color and titratable acidity. MAP with both PE film and silicon window film could maintain higher TSS in the fruits, and delayed skin wrinkles. MAP with PE films was the most effective and could prolong the storage period of goji fruit from 21 to 35 days.

Chlorine Dioxide (ClO₂) is a new type of high-efficiency, safe, and non-toxic disinfectant that has attracted extensive attention in recent years. It has many functions, such as disinfection, sterilization, antisepsis, deodorization, freshness preservation, and bleaching. Exposure of goji fruits to 40 mg/mL ClO₂ could effectively control the decay rates and decrease of Vitamin C content, maintaining fruit quality, and prolonging the storage period (Li et al., 2011b) of goji berries.

Calcium Chloride (CaCl₂) could reduce the rate of respiration and ethylene production and increase the activity of SOD and POD, thus delaying the senescence of goji fruit in a certain period of time (Mao et al., 2011). The calcium ion (Ca^{2+}) is a secondary messenger that plays pivotal roles in regulating physiological functions in tissues of fruits and vegetables. Ca^{2+} has also been shown to positively influence membrane integrity. Apoplastic Ca^{2+} ions are required to bridge the phosphate and carboxylate groups of phospholipids and proteins on the plasma membrane surface,

maintaining proper membrane structure and function and reducing membrane leakage.

Among postharvest treatment applied also for goji there are some study referring to the hot water dipping. Hot water and hot ethanol (EtOH) individual or as combined treatment effectively decreased the rot rate of goji berry. The incidence of the rot rates for hot water- and hot EtOH-treated fruits were 66.7% and 50.5% lower than the control, respectively, after 28 days of the storage. However, the weight loss of fruit treated with hot water and EtOH alone was higher than that of the control and also had a lower acceptance due to some off-taste, discoloration, less juiciness, and softening (Wei et al., 2013).

4.4. Plant Extracts as Alternative Preservatives

Natural bioactive plant extracts a have the advantage of non-pollution and non-toxicity attracting the interest of the research in recent years. Lotus leaves contain a variety of alkaloids and flavonoids, which have strong antioxidant and bacteriostatic effects, and can be used as food additives and preservatives. Lemon oil, an aromatic oil extracted from lemon fruit or peel, is rich in flavonoids and other components with significant antioxidant activity, which can be used in food and pharmaceutical industry. Zhao et al. (2014) tested the effects of 18 plant extracts on the storability of the fresh goji fruits, concluding that 2 g/L lotus leaf extract in EtOH and 400 μ L/L lemon oil extract were the most effective, and achieved a marketable fruit rate of above 80% after 5 days of storage at 20°C.

In vitro experiments showed that carvacrol treatments could significantly inhibit the spore germination and mycelial growth of *A. alternata*, a black mold pathogen of goji fruit, and the effectiveness was dose dependent. The inhibition rates of carvacrol at 8μ L/L on the mycelial growth and spore germination were 80.81% and 57.17%, respectively. Less mycelial, rough surfaces, non-uniform thickness, and distorted

cells were observed under scanning electron microscope. An *in vivo* test confirmed that carvacrol treatment could inhibit the incidence of black mold disease and enhance the activities of POD and phenylalanine ammonia lyase (PAL) (Wang et al., 2019a).

The inhibitory effects of potato glycoalkaloids (TGA) on four fungal pathogens isolated from goji fruits were studied, with the best effects on *Fusarium sp*. TGA at 0.15 g/mL which significantly inhibited the activity of defense enzymes, including CAT, PPO, POD, PAL, SOD, and chitinase (CHT) and helped to maintain the contents of Vit C, phenolics, lignin, flavonoids, and proline, and ultimately significantly reduced the disease index of harvested goji fruit (Chen et al., 2018).

5. Assessing Quality of Small Fruit

Sorting small fruit such as goji to remove defective berries is a labor-intensive task that is usually done by hand. Manual sorting is affected by subjective and physiological issues such as weariness, personal habits, and environmental factors such as light, belt speed, and general working circumstances, as do other manual processes. For small fruit these difficulties are even more critical due to the reduced dimensions of fruit and eventual defects which may also be frequently hidden in the side laying on the conveyor belt, which to the other side, increase the number of fruit to be controlled per time unit. In addition, defects at the early stages are barely visible, even if a stationary belt. To avoid these shortcomings, automated and objective optical approaches that have been effectively employed for quality assessment and trustworthy inspection of fresh horticultural commodities.

In the food industry, different online and offline digital image processing approaches have been employed to assess quality. When it comes to large-scale, continuous food quality monitoring, online image techniques are more popular, such as using a conveyor belt and an image capturing module to collect a constant stream of

photographs (Wang et al., 2018). Offline techniques entail categorization based on individual photos acquired from batches of samples, which may then be translated for mass use through consumer applications (Song, Jiang, Wang, & Vincent, 2020). In addition to digital camera, Visible and Near Infrared (NIR) sensors or hyperspectral camera may be used. Visible and near-infrared spectroscopy, in particular, are a well-established technique for determining chemical contents in food, and are used online for the prediction of soluble solid content or other maturity-related index as titratable acidity and firmness Hyperspectral imaging (HSI) is a combination or integration of imaging and spectroscopy and is used for the quantitative prediction of physical and chemical characteristics of the food samples as well as their spatial distribution. Hyperspectral cameras in the VIS and VIS-NIR range are therefore used, for sorting defective fruit, grading for dimensions, maturity and quality, and can provide at the same time, multiple information on internal quality composition and maturity stage of each individual fruit.

5.1. Digital Imaging

An image capturing system typically includes a camera module and a computer sustem s. The most frequent method is to use Charged Coupled Devices (CCD) cameras to capture RGB (Red, Green, Blue) color images. To extract relevant information, RGB color space can be transformed to HSV (Hue Saturation Value) or CIELAB (device-independent color space; L*, a*, b*, C*ab and Hab) color spaces (Stinco et al., 2013). Standard illumination systems are employed to provide steady light conditions during the image acquisition procedure. The next step is preprocessing, which entails removing noise from the input image and segmenting the region of interests (ROI) from the backdrop. In most cases, intensity thresholding and linked component analysis are utilized to segment ROIs. Once the ROI is obtained, we may extract desired features (variables), using several image processing techniques, giving relevant information of the images. These include

features based on color, texture, morphological features or geometry of food items. Color features generally include statistical parameters relevant to the color channels in the input image. Normalized histograms are also useful. The Local Binary Pattern (LBP), Noise Reductant Local Binary Pattern (NRLBP), Completed Local Binary Pattern (CLBP) or the Gray Level Co-occurrence Matrix (GLCM) are generally used to obtain the textural information from input images. Geometric features include parameters related to the shape of the food item, such as area, perimeter, length, width, aspect ratio and various shape factors. Several researchers have reported on the application of digital image for the assessment of fruit and vegetable quality, including morphological index as for tomato (Zaborowicz et al., 2017), and potato (Si et al., 2017); maturity index as for banana (Surya Prabha & Satheesh Kumar, 2015), papaya (Pereira et al., 2018)..., and orange (Jhawar, 2016), also classified for size sorting for defects as in apple (Blasco et al., 2003), and oranges (Rong et al., 2017) and detecting browning in fresh-cut artichoke (Amodio et al., 2011). Additionally, in the same cases color attributes provide information on internal composition, as found for red color of tomato's skin which correlated to the level of lycopene content of the fruit (Stinco et al., 2013). Estimation of single leaf chlorophyll content in sugar beet using RGB information of digital image (Moghaddam et al., 2011), and quantitative evaluation and prediction of anthocyanin content by using B/G color channel in lettuce leafe (Yang et al., 2016).

5.2. Spectroscopy

Sir Isaac Newton described the concept of dispersion of light and the optomechanical hardware of a spectrometer after passing light through a prism and observing the splitting of light into colors in 1665, when he described the concept of dispersion of light and the optomechanical hardware of a spectrometer. The different frequencies of the radiation exiting the sample are separated using gratings in these instruments. The collection of spectra and the construction of a calibration equation

to match this spectral data to the quality trait of a product obtained using standard laboratory methods are required for the development of chemometric methodology for assessing quality in a nondestructive way. This is known as a calibration equation in NIR quantitative analysis. The quality of the reference values associated with the samples in the calibration set makes a big difference in whether we succeed or fail at this activity. Nonetheless, once this stage of learning is completed, the ultimate result is likely to be near to that of an optimal analytical approach (Pieris et al., 1999). Spectroscopic methods, in general, provide detailed fingerprints of the biological sample to be analyzed based on physical characteristics of electromagnetic radiation's interaction with the sample material, such as reflectance, transmittance, absorbance, phosphorescence, fluorescence, and radioactive decay. The interaction of electromagnetic radiation with atoms and molecules is exploited in spectroscopic analysis to offer qualitative and quantitative chemical and physical information contained within the wavelength spectrum that is either absorbed or emitted. NIR spectroscopy is one of the most successful spectroscopic techniques used in the food sector. The absorption of electromagnetic light in the wavelength range of 780–2500 nm is the basis of NIR spectroscopy (Huang et al., 2008). Overtones and combination bands of O-H, N-H, C-H, and S-H stretching and bending vibrations produce the absorption bands seen in this spectral range, which enable qualitative and quantitative assessment of chemical and physical features.

As a result, NIR might be used to study all organic substances with O–H bonds (such as moisture, carbohydrate, and fat), C–H bonds (such as organic compounds and petroleum derivatives), and N–H bonds (such as proteins and amino acids). Some frequencies will be absorbed in a specific wavelength range, while others (that do not match any of the energy disparities between vibration response energy levels for that molecule) will be partially absorbed. The absorption spectra of a substance or sample are made up of this complicated relationship between absorption intensity and wavelength (Pasquini, 2003).

Indeed, modern NIR spectroscopy techniques necessitate a low-noise spectrometer,

computerized spectrometer and data capture, and data analysis using multivariate mathematics and statistical computer methods. In the application for fruits and vegetables, NIR source which is usually Xenon lamp is used to irradiate the sample; when the irradiation goes through the sample, it is absorbed and scattered, changing the sample's spectral properties (McClure 2003; Cozzolino et al., 2006; Nicolai et al., 2007). The structure of the sample, its moisture content, particle size, sample temperature, and, most significantly, its chemical content can influence this transformation. Irradiation scattering in fruits and vegetables is induced by cell wall surfaces and suspended particles (mitochondria, chloroplasts, and starch granules) Nicolai et al., 2007). The phenomenon of energy absorption in the visible and NIR areas must be understood in order to grasp the idea of NIR. Figure 1 depicts the electromagnetic radiation spectrum in the visible, near-infrared, and mid-infrared regions.

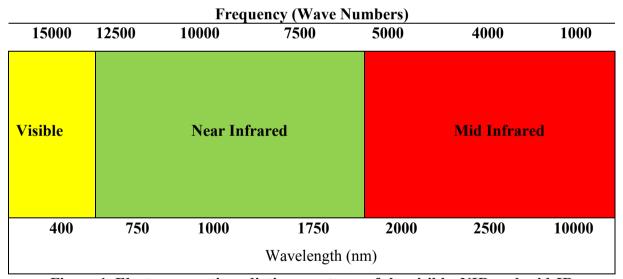


Figure 1. Electromagnetic radiation spectrum of the visible, NIR and mid-IR region.

Most light energy penetrates only a very short distance and exits near the point of entry; this is the basis for color. However, some penetrates deeper into the tissues and is altered by differential absorbance of various wavelengths before exiting and

therefore contains useful chemometric information. Such light may be called diffuse reflectance, body reflectance, diffuse transmittance, body transmittance or interactance (Abbott, 1999). Meanwhile, the interactions of constituents within product cells alter the characteristic absorbance wavelength and cause many overlapping absorbances (Park et al., 2002). In an attempt to determine the light penetration depth in fruit tissue for each wavelength in the range from 500 to 1900 nm, Lammertyn et al. (2000) found that the penetration depth in apple fruit is wavelength-dependent: up to 4 mm in the 700–900 nm range and between 2 and 3 mm in the 900–1900 nm range.

5.3. Hyperspectral Imaging

Hyperspectral imaging couple's spectral analysis with imaging providing the spatial distribution of a feature (chemical compound, defect) and the spectral information representing the response of the acquired pixel based on tissue structure and composition. The hyperspectral imaging system consists of five basic components: a camera with a cooled two-dimensional (2D) light detector, a spectrograph, a translation stage, illumination units, and a computer (Kim et al., 2002; Polder et al., 2002). Each of these components has its own features that affect the system's overall accuracy. To characterize the overall system's performance. The optimal illumination, for example, should be uniform over a vast region with no radiation damage to the samples. The hyperspectral imaging system's wavelength dispersion unit is essentially a grating spectrograph with a 2D detector array. It makes use of a field-limiting entrance slit and an imaging spectrometer with a dispersive element to enable the 2D detector to sample both the spectral and spatial dimensions at the same time. The light is focused on an entry slit by the imaging lens, then collimated, scattered by a grating, then focused on the detector by a grating. The camera's field of vision is often moved (or scanned) to provide the second spatial dimension. Both the slit width and the optical aberration affect the system's spectral resolution. When light enters the spectrograph, it is distributed into different directions based on wavelength while maintaining its spatial information. The dispersed light is then projected onto the detector array, yielding a two-dimensional image with one dimension representing the spectral axis and the other holding the scanning line's spatial information. The object to be measured is illuminated by a light source, such as a halogen lamp, and the entrance optics, such as a camera lens, gathers the radiation from the object and generates an image on the image plane, which is where the imaging spectrograph's entry slit is positioned. The slit serves as a field stop for determining the instantaneous FOV in terms of spatial area. The slit's radiation is collimated by a lens or a mirror, then dispersed by a dispersing device, which is generally a prism or grating, so that the radiation's propagation direction is determined by its wavelength. The focused radiation is detected by a 2D detector array such as charge-coupled device (CCD) or a complementary metal-oxidesemiconductor (CMOS) detector. A 2D detector array may sample one spatial dimension and the spectral dimension of a 3D cube concurrently using the image spectrograph. Slit width regulates the amount of light entering the spectrograph in addition to defining spectral resolution. The collimator also makes this light parallel so that it can be dispersed by the disperser. The object's second spatial dimension is created by scanning or shifting the instrument's FOV relative to the scene, which corresponds to the positions. Hyperspectral image is, in fact, made up of many congruent images that indicate intensities at various wavelength bands and are made up of vector pixels (voxels) that contain both two-dimensional spatial information (m rows and n columns) and spectral information. A three-dimensional hyperspectral cube, also known as a hypercube, data cube, data volume, spectral cube, or spectral volume, is a collection of data that can reveal physical and/or chemical information on a material under test (Cogdill et al., 2004). Physical and geometric observations of size, orientation, shape, color, and texture, as well as chemical/molecular information such as water, fat, proteins, and other hydrogenbonded elements, can all be included in this data (Lawrence et al., 2003). However, combining these two characteristics (spectral and spatial) is not straightforward, owing to the fact that it necessitates the creation of a three-dimensional (3D) data collection including multiple photographs of the same object, each taken at a different wavelength. Pixels may be written as numbers since they are digitalized gray values or intensities at a specific wavelength. At one wavelength, the intensity levels of a spatial image in the hypercube may have 8-bit gray values, where 0 represents black and 255 represents white. In more precise systems, the intensity values of each pixel having 12-bit (212 gradations, i.e., 0–4095), 14-bit (214 gradations, i.e., 0–16383) or 16-bit (216 gradations, i.e., 0–65535) gray levels are used. For many applications, 12-bit dynamic range is adequate and can provide high frame rates. For more demanding scientific applications such as cell, fluorescence or Raman imaging, a higher performance 16-bit cooled camera may be advantageous.

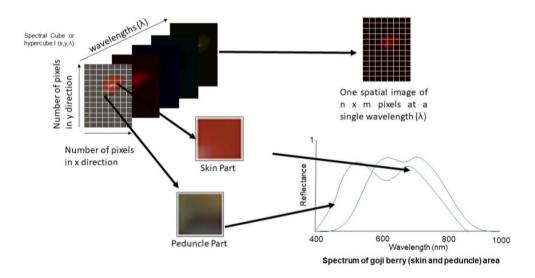


Figure 2. Schematic diagram of hyperspectral image (hypercube) of goji berry fruit showing the relationship between spectral and spatial dimensions. Every pixel in the hyperspectral image is represented by an individual spectrum containing information about chemical composition of this pixel.

Figure 2 shows one example of a hypercube generated from a hyperspectral acquisition of the goji berry fruit. Each sub-image in the raw hyperspectral image

indicates the intensity and spatial distribution of the reflectance of tested object at a certain waveband. All individual spatial images could be picked up from the hypercube at any wavelength(s) covering the spectral sensitivity of the system. Therefore, a hyperspectral image described as I (x, y, λ) can be viewed either as a separate spatial image I (x, y) at each wavelength (λ), or as a spectrum I (λ) at every pixel (x, y). Each pixel in a hyperspectral image contains the spectrum of that specific position. The resulting spectrum acts like a fingerprint which can be used to characterize the composition of that particular pixel. Since hyperspectral imaging acquires spatially distributed spectral responses at pixel levels, this allows flexible selection of any regions of interest on a target object, e.g. variable sizes and locations. For instance, if two different pixels from two different compositional locations in the hypercube are extracted, they will show different fingerprints or different spectral signatures. Therefore, without any further manipulation or preprocessing treatments of these spectral data, the difference in spectral signatures between peduncle pixel and skin pixel of the tested goji berry fruit shown in Figure 2, are noticeably distinguished. Hyperspectral imaging allowed users to discriminate the harvest time and to predict the internal content of soluble solids, phenols, and antioxidant activity of fennels (Amodio et al., 2017), allowing them to create of a concentration map for each component. Furthermore, recently in dried black goji berry, Zhang et al. 2020 successfully predicted the total anthocyanin, total flavonoid, and total phenols by using the hyperspectral image method, and Arslan et al. (2018) successfully predicted antioxidant activity on dried black goji berry. Additionally, for the maturity assessments, the successful use of hyperspectral technique is reported, as the discrimination of mature and immature-green tomatoes (Fatchurrahman et al., 2020) using fluorescence, and for the discrimination of different maturity classes as for okra (Xuan et al., 2021), grapes (Benelli et al., 2021), cherry (Li et al., 2018), and apples (wang et al., 2022).

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General objectives

The objective of this thesis attempted to increase available information on the postharvest characterization and metabolic behavior of goji berry fruit during ripening and storage in order to improve postharvest handling and technology. Due the scarce information available, the consumption of goji berry is limited to the production area while due to its nutritional properties and pleasant taste, a wider diffusion is highly advisable. Thus, we described the objectives as follow:

- 1. Postharvest characterization of Goji Berry (*Lycium barbarum* L.) at different development and maturity stages also in term of metabolic behavior, for its classification as climateric or non climateric fruit;
- 2. Effect of low storage temperature on postharvest physiology and quality of Goji Berry (*Lycium barbarum* L.) in order to assess its sensitivity to low temperature and the optimal storage temperature;
- 3. Effect of controlled atmosphere with high carbon dioxide on quality of goji berry fruits (*Lycium Barbarum* L.);
- 4. Potentiality of non-destructive method based on of Visible-NIR a Hyperspectral Imaging for sorting defective goji berry fruit (*Lycium barbarum* L.);
- 5. Potentiality of non-destructive method based on of Visible-NIR and Near-Infrared Hyperspectral Imaging for the Prediction of internal composition of Goji Berry (Lycium barbarum L.).

PART II: EXPERIMENTAL

Characterization and Postharvest Behavior of Goji Berry

(Lycium barbarum L.) During Ripening

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ABSTRACT

This study aimed to characterize goji berry (Lycium barbarum L.) fruit across different stages of maturity and ripening in terms of color, firmness, phytochemicals, and metabolic behavior. According to the producer's indication, the goji berry fruit was divided into six classes: early immature (green) to fully ripe (full red). Several maturity indexes were monitored for all classes, including dimension, weight, color (hue angle), firmness, soluble solid content (SSC), pH, and titratable acidity (TA). Fruit dimensions on the plant increased from class 1 to 6 starting from 8.08 mm in length, 3.95 mm in width, and 0.07 g of weight, to 16.26 mm, 13.15 mm, and 1.29 g, respectively. Soluble solids increased from 2.68 % to 23.5 %; the highest value observed even after storage. Goji berries showed a rise in respiration rate and ethylene production in the early stages of development. Goji berry stored for 8 d at 25 °C showed significant changes in color, soluble solids, TA, respiration, and ethylene production. Soluble solids from class 5 stabilized around the maximum value of 23 % after eight days of storage at room temperature. Their high nutritional value was confirmed by the content of vitamin C, which is comparable to that of citrus fruit. It reached the maximum value of 0.52 g/kg at full ripening, whereas the phenolic content decreased during ripening to values of 2.15 g/kg. The latest contributed considerably to the high antioxidant content of the berries. Results obtained in this study contribute to better understand the postharvest behavior of goji fruits enabling a clearer definition of quality attributes during ripening and, in turn, improving postharvest handling and distribution of goji berries as fresh fruit.

Keywords: Goji berry, respiration rate, ethylene production, maturity index, physicochemical changes, ripening

1. Introduction

Goji berry (*Lycium barbarum* L.), also known as "Wolfberry," is native to China and is one of the most common members of the Solanaceae family (Jatoi et al., 2018). It is widely known for its high antioxidant compounds, primarily phenolics (Yao et al., 2011). Diet including polyphenolics and total antioxidants from goji berries benefits vision, diabetes, kidney, liver, weight control, anti-cancer, and antiaging (Kulczyński and Gramza-Michałowska, 2016; Luo et al., 2004). Among phenolics, cinnamic acid is the most abundant (461.14 mg/100 g FW), followed by ferulic acid (125.80 mg/100 g FW), catechin (347.94 mg/100 g FW), and flavonols in the form of hyperoside (116.27 mg/ 100 g FW), and rutin (5646.7 μg/g pm) (Mocan et al., 2014; Donno et al., 2015). Polysaccharides are the most abundant compounds in the total dry matter of the fruit. Furthermore, goji berries are rich in vitamin C, with a reported concentration of 49 mg/100 g (Donno et al., 2015).

Fruit ripening is a complex physiological process involving significant external and internal changes that influence the fruit's appearance, firmness, flavor, and aroma (Corpas and Palma, 2018; Pech et al., 2018). Understanding the dramatic metabolic processes during fruit ripening is crucial for fruit handling and management (Atta-Aly et al., 2000a). A dramatic increase in respiration during ripening, indicated by a rise in the production of CO₂, was proposed to be climacteric behavior. This is because fruit have been categorized as climacteric and non-climacteric based on their respiratory patterns (Biale, 1964). Furthermore, Atta-Aly et al. (2000b) reported that a negative ethylene feedback mechanism suggests non-climacteric behavior, while a positive feedback mechanism shows climacteric behavior during ripening. The ethylene production rate usually increases logarithmically.

From a pomology point of view, goji berry's first fruition is typically observed in 3-year-old plants (Kulczyński and Gramza-Michałowska, 2016). The mature fruit is 1

to 2 cm long, with an ellipsoid shape and a bright orange-red color similar to mature mini-tomato, and contains 20–40 tiny seeds per fruit. It is sweet and has a tangy aroma (Jatoi et al., 2018). Kafkas et al. (2021) reported that the green stage is the most appropriate for extracting organic acids for pharmacological purposes. The red stage is processed into dried fruit for direct consumption, benefiting from sugars and L-ascorbic acid content. There is no indication of its use as a fresh fruit. Ascorbic acid is thermally sensitive (Badin et al., 2021), and therefore, the consumption of fresh goji berries should be highly recommended. However, there is no clear information regarding the characterization of fruit maturation stages, and the classification in terms of the metabolic behavior of fruit has not been explored. Therefore, this study aimed to characterize the goji berry maturation stages and metabolic behavior for the correct management of harvest and postharvest storage, thereby promoting its fresh consumption.

2. Materials and Method

2.1. Sample Preparation

A total of 1.5 kg of goji berry fruit (*Lycium barbarum* L.; Cultivar: sweet berry), grown in an open field in Castellaneta, (province of Taranto, Italy), was harvested by picking the fruit with its peduncle. Damaged fruit were removed, leaving 1.3 kg of healthy fruit in all maturation stages from green to red. Additionally, 20 branches were randomly removed from 20 4-year-old adult goji berry plants, for the evaluation of the frequency distribution of the fruit classes on the plant. Fruit were classified according to 6 classes according to grower indications, differing in size and skin colors. Physical, chemical, and metabolic indicators, were measured at harvest for each class, and we assume their changes from the least to the most mature well depicted the evolution of maturity indicators, while on the plant. Fruit dimension, weight, firmness, soluble solid content (SSC), titratable acidity (TA), and pH were measured on 20 replicates of about 8 g each, except for classes 1 and

2 of approximately 6.5 g each, for which less fruit were available.

An additional 60 healthy fruit from all six classes (10 fruits per class), based on the physical aspects of color, weight, size, and shape, were used to assess respiration rate and ethylene production at ambient temperature. The remaining fruit (about 0.4 kg) were divided into 10 replicates and stored for eight days at 25 °C to assess color, SSC, and TA changes during ripening.

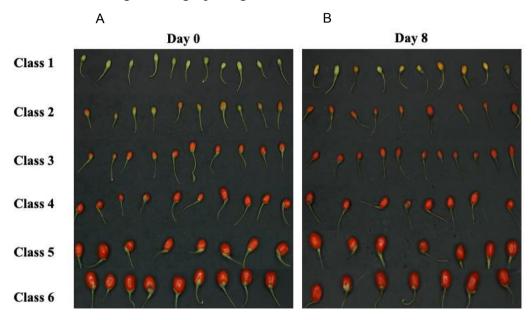


Figure 1. Fruit appearance for the 6 different classes at harvest (A) and after 8 d of storage at 25 °C (B).

Figure 1A represents a selection of goji berry fruit assorted in the six classes. The fruit showed differences in color and size, starting from small, green fruit with the size growing and the color turning into orange and finally red, as they go from calls 1 to class 6.

2.2. Physical attributes

Goji berries were weighed at harvest using a digital electronic balance (Shimadzu, Japan), and the dimensions of the fruit were measured using digital calipers (Japan Mitutoyo 500-197-30).

The color of the goji berry fruit was measured by extracting one image consisting of five fruit per replicate (20 images) taken by a spectral scanner (DV s.r.l., Italia), at harvest and after 8 d of storage. Color changes were quantified in the CIE L*a*b* color space. Hue° = arctg $\frac{b}{a*}$ was calculated from the a* and b* values. Firmness was assessed at harvest by using a single fruit for each replicate. The fruit was compressed between two parallel plates using an Instron Universal Testing Machine (model 3340) at a crosshead speed of 75 mm min⁻¹. The firmness of the fruit samples was defined as the rupture load of the deformation curve and expressed in Newton (N).

2.3. Chemical

2.3.1. Soluble Solids Content, Titratable Acidity, and pH

At time 0 and eight days after storage, approximately 2.5 g of goji berries per replicate were placed in a falcon tube, homogenized with an Ultra-Turrax (IKA T18 basic, Germany), then filtered through two layers of cheesecloth (JC NONSTE SWAB 4040, China). The filtrate was used to determine the SSC (%) using a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan). TA and pH were measured using 1 g of filtrate in an automatic titrator (TitroMatic CRISON, Barcelona, Spain). The samples were titrated with a 0.1 mol L⁻¹ NaOH solution up to a final pH of 8.1 and were expressed as a percentage of citric acid per 100 g sample.

2.3.2. Determination of Ascorbic Acid, Dehydroascorbic Acid, and Vitamin C

For each replicate, the concentrations were assessed by homogenizing 2.5 g of fruit tissue with an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min in 3 mL of methanol/water (5:95), citric acid (21 g L⁻¹), EDTA (0.5 g L⁻¹), and NaF (0.168 g L⁻¹). Due to technical challenges caused by the low concentration of vitamin C, Dehydroascorbic Acid (DHAA), and Ascorbic Acid (AA), stages 1 and 2 were excluded from the evaluation. The homogenate was filtered through cheesecloth, and the pH was adjusted to 2.2-2.4 with 6 mol L⁻¹ HCl. The filtrate was centrifuged at 12000 rpm for 10 min. The supernatant was recovered, filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA), and then through a 0.2 µm cellulose acetate filter. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by (Zapata & Dufour, 1992) with some modifications. HPLC analysis was performed after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxy ethyl) furol [3,4-b] quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 µL) were analyzed using an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separation of DFQ and AA was achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H₂O (5:95 v/v) containing 5 mmol L⁻¹ cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min⁻¹. The detector wavelengths were 348 nm for DHAA and 251 nm for AA. AA and DHAA contents were expressed as gram per kg of fresh weight.

2.3.3. Total Polyphenol and Antioxidant Activity

For each replicate, the total phenol content was determined by homogenizing 2.5 g of goji berries with an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min in 80 % methanol, 20 % water, and 2 mmol L^{-1} sodium fluoride. The homogenate was then centrifuged at 9000 rpm for 10 min at 4 °C. The method

described by Derossi et al. (2016) was used with some modifications. Up to $100~\mu L$ extract was mixed with 1.58 mL water, $100~\mu L$ of Folin–Ciocalteu reagent, and $300~\mu L$ of sodium carbonate solution ($200~g~L^{-1}$). Two hours after the extraction, the absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). The total phenol content was calculated using a calibration curve of gallic acid and was expressed as milligrams of gallic acid per 100~g of fresh weight (mg GA $100~g^{-1}$). The antioxidant assay was performed using the procedure described by Brand-Williams et al. (1995) with minor modifications. Fifty microliters of diluted sample were added to 0.950~mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after a 24 h incubation. Trolox was used as a standard, and the antioxidant activity was reported in g of Trolox equivalents per kg of fresh weight (g TE kg $^{-1}$).

2.4. Respiration Rate and Ethylene Production

For the assessment of metabolic behavior, the respiration rate of single goji berry fruits was measured under static conditions at ambient temperature (25 °C) following the protocol described by Kader (2002) with some modifications. Five replicate samples (individual fruit) for each maturity class, were placed in 10 mL sealed glass jars fitted with plastic tubes for gas sampling. The jars were closed, and after an acclimation time of 0.5 h, gas samples were removed. After each measurement, residual CO₂ was removed by blowing with a pipette, and the jars were left open until the next measurement. Measurements were done at time 0 h, which was around 7 hours after harvest, and after 3.3, 5.6, 23, 26, 28.3, 47, 95, 97.8, 99.8, 120.3, 145, and 168 hours. Gas samples (1 mL) were collected from each jar after the required time to accumulate CO₂ in the headspace up to a concentration of 0.1–0.2 % and injected into a gas chromatograph (Shimadzu, model 17 A, Kyoto, Japan) equipped with a thermal conductivity detector (200 °C). Separation of CO₂ was achieved on a Carboxen 1006 plot (30 m × 0.53 mm, Supelco, Bellefonte, PA,

USA), with a column flow of 7 mL min⁻¹ and an oven temperature of 180 °C. The calculation of respiration rate was based on the difference in concentration, sample weight, and free volume inside the jar (Caleb et al., 2012).

Ethylene production (μL C₂H₄/kg/h) was measured for five individual fruit for each class, using the same closed system described for CO₂ accumulation (Kader, 1992; Rinaldi et al., 2010). The accumulation of C₂H₄ in the headspace of sealed jars was measured taking 1.5 mL gas samples, which were injected into a gas chromatograph (Agilent, model 7890 A), equipped with a flame ionization detector; column Carboxen 1006 plot (30 m × 0.53 mm, Supelco, Bellefonte, PA, USA), with a column flow of 7 mL min⁻¹, and an oven temperature of 180 °C. The ethylene concentration was then applied to the sample weight, the free volume in the jar, and the elapsed time. The measurements were performed at time 0 (i.e., 27 h after harvest), and after 28.5, 53, 100, 125, and 168 hours, due to the long time necessary for ethylene accumulation (about 24 hours to have a detectable peak, with a sensitivity of 0.01 ppm).

3. Result and Discussion

3.1. Characterization of the 6 classes at harvest

3.1.1. Dimensions, maturity index, and metabolic activity

Figure 2 shows goji berry fruit from different development and ripeness stages on the main branch. Since the plants continuously produce new blossoms, resulting in the non-homogenous stages from green (stage 1) to red (stage 6) on the same branches. Figure 3 depicts the distribution of the different stages on the branch during the harvest period. Green fruit at stage 1 was dominant in terms of the number of fruit in the branch (48 % of the total fruit) compared to stage 4 or 5, which are ready to be harvested (15 % and 11 % of the total fruit distribution). The yield reached a density of 1.07 fruit/ cm with the SD \pm 0.16 and SE \pm 0.04.



Fig 2. Example of fruit branches (Branch length = 51.75 cm; n = 20; SD = 8.19; SE = 1.83).

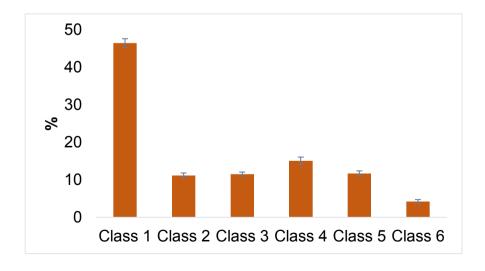


Fig 3. Frequency distribution over development and ripening stages of goji berry fruit (N = 1102) from 20 branches.

Fruit from the 6 different classes were characterized at harvest in terms of color and dimensions, firmness, and metabolic rate. Color and dimensions considerably changed with fruit development and ripening on the plant. In Figure 4, changes in several indexes can be observed for fruits belonging to each maturity stage over a storage time of 8 d. Color of fruit kept on the plant, gradually changed from green (Class 1) with a hue value of 89.61 ± 0.74 to orange, pink, and finally red with a hue value of 32.98 ± 1.95 for class 6, as the fruit increased in size. Color changes occurring during fruit growing are not very common, since many fruit as tomatoes, become red only after reaching maximum dimension. Distinctive differences were observed among classes regarding weight, width, and length. In class 1, the fruit weight was approximately 0.07 ± 0.019 g and gradually increased to class 6, reaching a weight of approximately 1.29 g ± 0.188 . The same trend was observed for fruit width and length (Table 1). The width and length increased from 3.95 mm ± 0.430 and 8.08 mm ± 0.98 to 13.15 mm ± 1.035 and 16.26 ± 1.049 for classes 1 and 6, respectively.

Regarding the color changes from green to red, in many fruit belonging to the same family, including tomatoes, chlorophyll degrades during ripening while secondary metabolites such as carotenoids accumulate (Ho and White, 2005; Lai et al., 2007). Carotenoids contribute to the bright red-orange color in fresh goji berries (Zhang et al., 2016), containing up to 321.09 μ g/g β -carotene (Jatoi et al., 2017). Additionally, it has been reported that among the types of β -carotene, the content of zeaxanthin esters in ripening goji fruit can exceed 77.5 % of β CE/g FW with zeaxanthin palmitate is especially abundant, comprising 31 %–56 % of the mg β CE/g FW (Peng et al., 2005). Regarding different colors in the ripening stages, to the best of our knowledge, there is only one report classifying the maturity level of goji berries into three categories (green, unripe, and ripe) with a hue value of 86.4 for the green stage and 47.8 for the unripe and ripe stages (Kafkas et al., 2021). However, in this study, we reported an earlier green stage with a higher hue value of 89.6 (Figure 3), compared to the previous study that referred to a greenish-yellow color.

Furthermore, the colors of the unripe and ripe stages were comparable to those of classes 2 and 3 in our study, corresponding to an orange color and not to full red fruit. Our results agree with Jatoi et al. (2017), who reported the ripe fresh goji berry with a hue value of approximately 33. Regarding the dimensions, the same result of weight, width, and length at the green stage was reported in a previous study (Kafkas et al., 2021). However, concerning the literature on ripe *L. barbarum*, different results were reported for mature fruit weight, width, and length ranging from 0.3 g - 1.32 g, 6.98 mm – 12 mm, and 10.01 mm – 21.5 mm, respectively (Kafkas et al., 2021). Besides the differences due to the ecology, planting methods, and production techniques, this variability confirms the lack of a clear definition of the maturity index for this fruit.

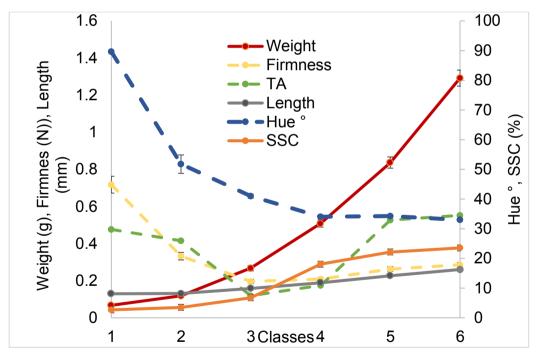


Figure 4. Evolution over the different classes of the physical indexes of goji berries at harvest.

Table 1. Maturity index distribution for goji berry fruit from 20 replicates, as classified destructively at harvest.

at harvest.										
		Peel Hue (°)	Weight (g)	Width (mm)	Length (mm)	TA (%)	рН	SSC (%)	Firmness (N)	
Class 1	Mean	89.61	0.070	3.95	8.08	0.476	5.353	2.675	0.717	
	SD	± 0.74	± 0.019	± 0.430	± 0.979	± 0.009	± 0.009	± 0.102	± 0.095	
	SE	± 0.13	± 0.003	$\pm \ 0.078$	± 0.179	$\pm~0.002$	± 0.002	± 0.023	± 0.018	
	Max	90.04	0.117	4.88	9.8	0.496	5.367	2.8	1.316	
	Min	86.62	0.044	3.2	5.85	0.466	5.319	2.5	0.264	
Class 2	Mean	51.74	0.12	5.02	8.15	0.415	5.280	3.520	0.332	
	SD	±17.04	± 0.025	± 0.612	± 0.863	± 0.001	± 0.045	± 0.111	± 0.111	
	SE	± 3.11	± 0.005	± 0.112	± 0.158	± 0.000	± 0.010	± 0.025	± 0.020	
	Max	88.36	0.166	6.07	9.7	0.420	5.367	3.7	0.560	
	Min	32.07	0.082	0.082	6.35	0.414	5.218	3.3	0.138	
Class 3	Mean	40.96	0.27	7.24	9.89	0.119	6.208	6.725	0.196	
	SD	± 3.62	± 0.064	$\pm \ 0.849$	± 0.982	± 0.006	$\pm \ 0.067$	± 0.150	± 0.059	
	SE	± 0.66	± 0.014	± 0.190	± 0.220	$\pm~0.003$	$\pm~0.034$	± 0.075	± 0.011	
	Max	47.00	0.362	9.2	11.81	0.215	6.276	7	0.357	
	Min	35.20	0.164	5.51	8.03	0.174	6.104	6	0.103	
Class 4	Mean	34.03	0.51	9.40	11.82	0.175	6.179	18.043	0.208	
	SD	± 1.49	± 0.085	± 0.694	± 1.242	$\pm~0.008$	± 0.055	± 0.500	± 0.048	
	SE	± 0.27	± 0.019	± 0.155	± 0.278	± 0.001	± 0.01	± 0.250	± 0.009	
	Max	38.06	0.695	10.86	14.76	0.190	6.287	19	0.323	
	Min	30.77	0.328	8.2	10.01	0.152	6.087	17	0.129	
Class 5	Mean	34.24	0.84	11.28	14.19	0.526	5.412	22.17	0.263	
	SD	± 1.30	± 0.132	± 0.779	± 1.229	± 0.012	± 0.464	± 0.747	± 0.068	
	SE	± 0.24	± 0.030	± 0.174	± 0.275	$\pm\ 0.002$	$\pm~0.008$	± 0.136	± 0.012	
	Max	37.12	1.112	12.51	15.68	0.541	5.448	23	0.468	
	Min	32.39	0.629	9.91	11.39	0.494	5.316	21	0.147	
Class 6	Mean	32.98	1.29	13.15	16.26	0.552	5.655	23.530	0.285	
	SD	± 1.95	± 0.188	± 1.035	± 1.049	± 0.019	$\pm~0.043$	± 0.507	± 0.071	
	SE	± 0.36	$\pm~0.042$	± 0.231	± 0.234	$\pm~0.003$	$\pm~0.008$	$\pm~0.093$	± 0.013	
	Max	37.69	1.711	15.58	19.35	0.579	5.746	24	0.459	
	Min	28.83	0.988	11.32	14.58	0.509	5.613	23	0.148	

Simultaneously with changes in color and dimensions, we observed an approximate 10-fold increase in SSC values from the green class to the ripest, with values of $2.675\% \pm 0.102$, $3.52\% \pm 0.11$, $6.725\% \pm 0.150$, $18.043\% \pm 0.5$, $22.17\% \pm 0.747$, and $23.53\% \pm 0.507$, respectively. A previous study on the green stage reported that SSC could reach around 6.17% - 7.17% (Kafkas et al., 2021), but the study harvested bigger fruit, corresponding to class 3 of our study. However, in the ripe class (i.e., classes 5 and 6), our results are in accordance with those of previous studies, which found that the SSC of *L. barbarum* varies from 21\% to 24\% (Xie et al., 2017; Zhao et al., 2020; Kosińska-Cagnazzo et al., 2017).

As for total acidity, our results were in agreement with those from previous studies, showing 0.5 % to 1.2 % or 0.2 % to 1.69 % citric acid depending on fruit genotypes and varieties (Kafkas et al., 2021; Zhao et al., 2015; Donno et al., 2015). pH value variation from 5.35 for class 1 to 5.66 for class 6, agrees with the results reported by Zhao et al. (2020).

Firmness is another important factor in fruit maturity and is one of the most critical quality aspects. Fruit of class 1 has the highest firmness value reaching 0.72, which was reduced to about 0.20 for the classes 3 and 4, and slightly increasing for the latest classes up to 0.28 N. To the best of our knowledge, no study has reported the firmness profile of the unripe stages of goji berry. There is some indication for ripe goji berries, but the data is not comparable because the authors used a fruit penetrometer with a diameter of 2 mm. In our opinion, that is not the best method due to the small size of the goji berry, which makes the use of a probe inaccurate (Zhou et al., 2020; Zhang et al., 2016).

As for respiration rate and ethylene production, early stages of development showed higher value than fully ripe fruit. In particular, a higher respiration rate was observed for class 2, followed by class 1, being 89.58, 65,77, and 52.82 mL/kg/h, respectively. Ethylene production decreased from class 1 (2.93 μ L C₂H₄/kg/h) to class 6 (0.24 μ L

C₂H₄/kg/h). The higher respiration and ethylene production in the early stage of development may be due to the energy requested for cell division and enlargement, as reported for many fruit such as mango, jujube, and olive ((Lakshminarayana et al., 1970; Abbas and Fandi, 2002; Kitsaki et al., 1999), but there is a lack of information about cell growing for goji.

Table 2. Respiration rate and ethylene production of goji berry at harvest.

	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
Respiration rate	65.77	89.58	52.82	29.64	42.98	24.93
$(mL CO_2/kg/h)$						
Ethylene production	2.93	1.81	1.75	0.70	0.43	0.24
$\mu L C_2H_4/kg/h$						

3.1.2. Chemical composition

Goji berries are commodities rich in vitamin C, which is beneficial for human health (González-Molina et al., 2010). Results of this study for the red classes are in accordance with Donno et al. (2015), indicating that goji berries contain around 0.4 g/kg, typical of citrus fruit (Figure 5). Due to the limitation of the sample extraction method and the scarcity of fruit for classes 1 and 2, we could not assess the vitamin C content for these classes, but we observed a linear increase of Vitamin C from class 3 (0.11 g/kg) to class 6.



Figure 5. AA and DHA (and Vit C as summation) of goji berries from class 3 to 6 at harvest.

The variety and abundance of phenolic compounds in fruit vary depending on geographic location, genetic variation, region, agricultural practices, cultivation method, year of harvest, growth period, and storage conditions (Dong et al., 2012). A study conducted by Islam et al. (2017) reported that the total phenol content of ripe goji berries varies between 2.17 ± 1 g/kg and 4.48 ± 1 g/kg, which is consistent with the results of this study. To the best of our knowledge, no study reported the total phenol of goji berry in the early developmental stage. However, this study detected a total phenol content of 3.35 ± 0.15 g/kg for class 3 that decreased to 2.1 ± 0.05 g/kg in class 6 (Figure 6). Due to the lack of samples in the early stages, we could not evaluate the content of fruit from classes 1 and 2. However, this may be of interest in future studies.

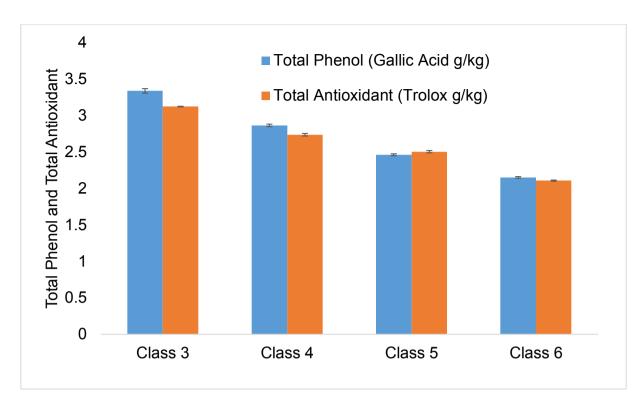


Figure 6. Evolution over the class of ripeness of AA and DHA (and Vit C as summation) of goji berries at harvest.

Similar to the phenol concentration, the total antioxidant content of goji berry fruit decreased from 3.12 ± 0.08 TE g/kg to 2.12 ± 0.08 TE g/kg from class 3 to 6. In class 6, goji berries contained the lowest total antioxidant level. To the best of our knowledge, no previous studies reported the level of total antioxidants over the development and maturity of goji fruit. A decrease in phenolic concentration and antioxidant activity and increased vitamin C during ripening were observed in tomatoes (Raffo et al., 2002). This trend is primarily related to genetic control factors and is probably linked to the decrease in flavonoids during ripening (Macheix et al., 2018.). Additionally, the increase in vitamin C content from 0.1 to 0.5 g/kg during ripening was not affecting the level of total antioxidant activity, which showed a 6 to 20 fold higher concentration but decreased from 3.1 to 2.1 g/kg with the advancing of ripening. This correlated with the phenolic content, indicating their contribution to the antioxidant activity. The antioxidant content in ripe fruit is similar to

previously reported values ranging from 2.2 to 2.8 TE g/kg (Jatoi et al., 2017) (Donno et al., 2015). Based on results from this study, goji berries should be consumed in the early stage of development and ripening for the antioxidant and total phenol benefits and in the ripe stages for the full fruit size and highest SSC, organic acids, and vitamin C levels.

3.2. Fruit quality changes after storage

Goji berry stored for 8 d at 25 °C showed significant changes in color, soluble solids, TA respiration, and ethylene production. Color changes are visible in Fig. 7, and detailed in terms of Hue angle which is showing how the red component is increasing over storage time for the first 3 classes, and that starting from class 3 when on the plant on the plant, and class 4 if detached, hue angles for all fruits converged to the same value (about 35 °).

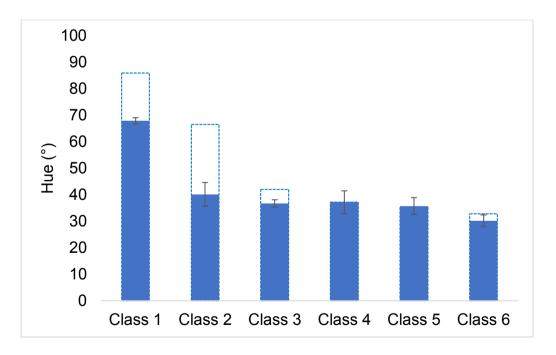


Figure 7. Color changes of goji berry from different classes after storage at 25 °C for 8 d (white bars with broken lines are used to indicate initial value at harvest).

Regarding SSC which is shown in Fig. 8, their level increased for all classes, except class 6, when fruit were detached from the plant, but as for color, early stages could not develop the maximum value of SSC. Fruit of class 5, after 8 d of storage reached the same value as fruit from class 6 at harvest (about 23 %), while fruit from class 4 showed the biggest increase in SSC when detached from the plant, but reaching only 18 °C. The opposite trend was observed in the TA that their level decreased for all classes, except class 3, this may suggest that the fruit are following senescence during storage.

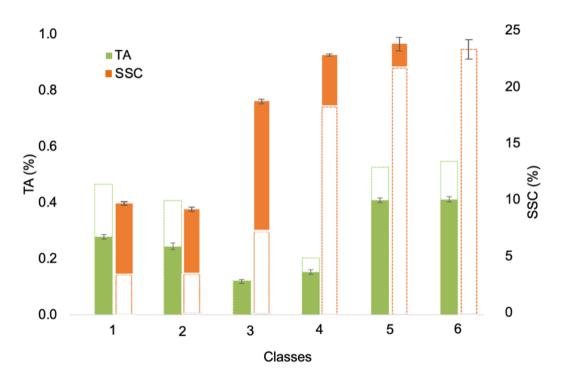
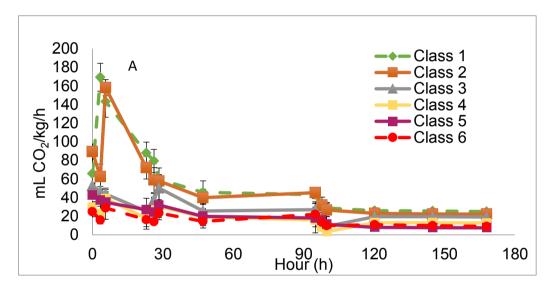


Figure 8. Changes of TA and SSC at harvest and after 8 d of storage at 25 °C (white bars with broken lines are used to indicate initial value at harvest).

Respiration rate and ethylene production are shown in Fig. 9. After harvest, the fruit supports active metabolism, as indicated by the rise in respiration rate and ethylene production for climacteric and the relatively stable respiration rate and ethylene production for non-climacteric fruit (Atta-Aly et al., 2000a; Boeckx et al., 2019). The increase in respiration, together with ethylene production, accelerates fruit

softening (Lèlievre et al., 1997). Class 1 and 2 have maximum peaks of respiration rate corresponding to 158.1 ± 15 mL $CO_2/kg/h$ and 162.3 ± 8.7 mL $CO_2/kg/h$ at hours 3.33 and 5.6, respectively (Fig. 9A). Furthermore, Figure 9 B shows that also for ethylene, a rise is observed for classes 1 and 2 (3.57 \pm 0.09 μ L $C_2H_4/kg/h$ and 3.98 ± 0.9 C_2H_4 , respectively) after 100 hours (4 d of storage), but considering the gap since the preceding measure, due to long time needed for the accumulation of a detectable amount of ethylene in the jars, we can't exclude that the peak started before this time, occurring between 53 and 100 hours after harvest.



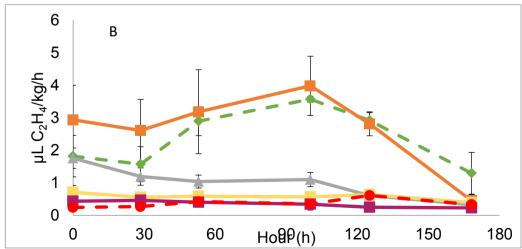


Figure 9. Postharvest evolution over the different classes of respiration rate (A) and ethylene production of goji berries during 8 d of storage at 25 °C (B)

The only rise in respiration rate and ethylene production was found in the early stage of fruit development (stages 1 and 2.) and this as previously indicated, has been reported for other fruit, such as peach (*Prunus persica* L. Batsch cv. Redhaven), showing an intense metabolism. Authors suggest that the evolution of a high rate of ethylene production in the pericarp and mesocarp was associated with an increase in ethylene forming enzyme (EFE) known as 1-aminocyclopropane-1carboxylic acid synthase (ACC) (Tonutti et al., 2019). Adams and Yang. (1979) reported that ACC is the immediate precursor of ethylene. Previous studies reported goji berry respiration and ethylene production rates of 0.08 mL CO₂/kg/h and 0.1 µL C₂H₄/kg/h, respectively, at 4 °C (Zhou et al., 2020). This lower value is in line with our results, considering that ethylene production and respiration rate are affected by the storage temperature (Fonseca et al., 2002) and that we measured respiration rate at 25 °C. Furthermore, there was a delay in the rise of ethylene with respect to CO₂ which for the climacteric peak is not very common since the respiration rate and ethylene production should be observed at the same time. However, this has been reported for some genotypes of nance fruit (Rivas-Castro et al., 2019) or mangosteen (Nochinda, 1992).

Although they showed a rise in respiration rate and ethylene production after harvest (Figures 9 A and B), the fruit of classes 1 and 2 only presented a small increase in SSC, and some color changes (figure 7); in addition, they did not show completion of their ontogeny as they never completed their increase in dimensions, as they would have if not detached from the plant. For this reason, and according to Watada et al. (1984), we cannot define these stages as physiologically mature, and we failed to detect climacteric evidence. For the other stages fruit did not show the same postharvest increase in respiration and ethylene production rates since they had probably taken place before harvest. Fruits from class 3 which were still orange colored at harvest completed color evolution in the following 8 days at room temperature, also showing a major increase in soluble solid content, although not

reaching the same value as if left on the plant. Fruit from stage 4 and on were already full red at harvest and presented major changes of soluble solids and titratable acidity during postharvest storage reaching values very similar to those of fruits at stage 6 which were considered fully ripe when picked from the plant. Their postharvest behavior suggested they might be climacteric but further evidence are needed, including the fruit response to exogenous ethylene.

Thus, considering all the maturation indexes (including dimensions), and chemical composition of goji berries of the considered classes, for the industrial practice purpose, given the consistent increase in size in the last stages those are probably the best time to harvest in order to maximize yield and SSC. Furthermore, due to the perishable nature of stage 6 fruit, we recommend harvesting at stage 5 or not earlier than stage 4. For stage 4 fruit, we observed maximum development of soluble solids after eight days of storage at room temperature, reaching a value of approximately 23 %. Based on information gained from the local producers, goji berry plants will start producing fruit two years after planting, and the optimal production will be reached when the plants are three to five years old. This yield will vary at around 1 to 3 kg per plant. The fruit is ready for harvest when the fruit reaches full color at stages 4 or 5. Fruit must be harvested by hand as they are not easily detached from the stem, and bruised fruit will quickly turn black. The fruition time starts in midsummer to late fall (Maughan and Black, 2015).

Conclusions

This study described, for the first time, the physical, chemical, and metabolic changes of goji berries at different developmental and maturity stages. Over the development and ripening process on the plant, we observed an increase in size, a decrease in acidity and firmness, an increase in soluble solids and vitamin C content, and a decrease in phenolic concentration and antioxidant activity. After 8 d at 25 °C

all stages went through color changes, from green to red, and showed an increase of SSC and a decrease of acidity, but only fruit from stage 4 developed full red color and soluble solid content, as dully red fruit of class 6, which remained unchanged over storage. Goji berry fruit showed a rise in respiration rate and ethylene production in the early stages of development (Stages 1 and 2), but no other peaks were observed to the latest stages when a climacteric is expected. Stating to these results, in order to achieve suitable fruit dimensions and yield and a potentially high content of soluble solids, we recommend harvesting at stage 5 and not earlier than stage 4. This information is crucial for postharvest fruit handling and promoting the consumption of fresh goji berries, thereby preserving and supplying a product with higher nutritional value compared to the dried and processed version.

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Author contribution:

DF: Conceptualization, Methodology, Software, Data curation, Formal analysis.

Writing - original draft, Writing - review & editing.

MLA: Conceptualization, Methodology, Data curation, Supervision, Writing - review & editing.

MLVDC: Methodology for chemical analysis, Formal analysis of Vitamin C.

LM: Methodology for chemical analysis, Formal analysis of Vitamin C.

GC: Conceptualization, Supervision, Writing - review & editing.

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Postharvest Physiology and Quality of Goji Berry (*Lycium barbarum* L.) Fruit Stored at Low Temperatures

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Abstract:

Goji berries are widely known for their outstanding nutritional and medicinal properties; they are usually found in the market as dried fruit or as juice because the fruit has a short shelf-life, and little information is available about its postharvest behavior at refrigeration temperatures. This study aimed to determine the optimal storage temperature for goji berry fruit by evaluating physicochemical and sensorial attributes during storage at three different temperatures (0, 5, and 7 °C) in a range that was not deeply studied before. The fruit respiration rate and ethylene production were measured along with the soluble solid content (SSC), pH, titratable acidity (TA), texture, color, electrolytic leakage, antioxidant activity, the incidence of chilling, mold damage, and vitamin C, total polyphenol, anthocyanin content. Fruit stored at 0 °C showed the lowest respiration rate and ethylene production (5.82 mL CO₂ kg⁻¹h⁻¹ and 0.667 µL C₂H₄ kg⁻¹h⁻¹, respectively); however, at this temperature, the incidence and severity of shriveling and dark spots and electrolytic leakage were the highest, reaching 59, 15.42 %, and 46 %, respectively. In contrast, 5 °C was found to be the best storage temperature for goji berry fruit; the fruit appeared fresh and healthy and had the highest scores during sensory analysis with a general acceptable impression, with the lowest damage attributable to chilling injury, 27.14 % fruit presenting shriveling, 6.17 % black spot damage, and 34 % electrolytic leakage on day 12 of storage. Storage of goji berries at 7 °C resulted in the lowest marketability and the highest incidence of decay. Significant differences were also found in the phytochemical attributes, vitamin C content, SSC, TA, SSC/TA ratio, total polyphenol content, DPPH, and anthocyanin content. This study broadens the existing knowledge of the postharvest behavior of fresh goji berries; our results can help improve the commercial life of goji berries and in ensuring high-quality attributes throughout distribution.

Key Words: Goji berries, shelf-life, storage temperature, sensorial attributes, freshness, chilling injury

1. Introduction

Wolfberry or goji berry fruit (Lycium barbarum L.), belonging to the family Solanaceae, is widely recognized for its exceptional health benefits (Jatoi et al., 2018). This shrub is native to Asia, primarily the central north region of Ningxia Hui (China), and was introduced to Europe in the 18th century given its excellent nutritive and medicinal value (Kulczyński & Gramza-Michałowska, 2016). Goji berry is particularly rich in nutraceutical compounds (carotenoids, flavonoids, phenolics, vitamins, and minerals) that exhibit anti-aging, antitumor, and antioxidant activities in the human body. Generally, goji berries are cooked and processed as tea, soups, or served with meat and vegetables. They are also utilized for juice, tincture, and wine production (Amagase and Farnsworth, 2011; Benzie and Wachtel-Galor, 2011; Potterat, 2010). The fruit is also consumed in dried form or processed in powdered form for medicinal purposes (Zhu & Zi, 1998). Given their high perishability, high water content, and susceptibility to damage and rot (Fan et al., 2019), fresh goji berries are generally available in areas where they are cultivated. This is also due to the lack of information on goji berry postharvest behavior and storage recommendations (Ling et al., 2020). Dipping in lecithin solution (Jatoi et al., 2017b) and edible coating applications based on lotus leaf extract (Fan et al., 2019) have been studied to prolong the shelf-life of goji. Ban et al. (2015) reported that mild heat treatment (40 °C for 30 min) combined with chitosan coating could delay decay occurrence on goji berries up to 28 days at 2 °C. Kafkaletou et al. (2017) tested instead CO₂-enriched atmospheres concluding that 15-20 % of carbon dioxide can help maintain the quality of stored goji berries for up to 14 days. The use of modified atmosphere packaging (MAP), studied by Palumbo et al. (2020), reaching an equilibrium of approximately 10 % CO₂ can help preserve berry weight losses and organoleptic quality while avoiding mold occurrence up to day 13 of storage at 7 °C. The postharvest ripening of goji berries has been controlled using salicylic acid treatment has also been reported, as this acid is used for inhibiting goji berry

postharvest decay (H. Zhang et al., 2021).

Regardless of postharvest technology, temperature and relative humidity are the first storage conditions to be accurately controlled to preserve quality and allow maximum shelf-life (Fatchurrahman et al., 2020; Mastrandrea et al., 2017). Studies on goji berry have only considered low (0 and 2 °C mostly) or high (10 or 20 °C) temperatures. Jatoi et al. (2018) reported the effect of temperature on postharvest quality of fresh goji berry fruit during storage at different temperatures (-2, 0, 10, and 20 °C) and concluded that 0 °C is the optimum temperature to maintain the berry phytochemical and sensory qualities. However, these results are not reliable because of the large temperature gap among the tested temperatures and not including the refrigeration temperature of 5 °C, which is used in the cold chain of fresh or minimally processed products for transport and sale.

As goji berries belong to the Solanaceae family, they are plausibly chilling-sensitive, such as tomato, bell pepper, and eggplant (Fatchurrahman et al., 2015; Tsouvaltzis et al., 2020); however, the quality of fruit stored at 0 °C may show a slower degradation compared with when stored at 10 or 20 °C (Derossi et al., 2016). Therefore, the objective of the present study was to compare the effects of different storage temperatures between 0 and 7 °C to identify the best storage temperature for maintaining goji berry quality and shelf-life, with particular attention to the occurrence of chilling injury symptoms.

2. Materials and Methods

2.1. Sample Preparation

Briefly, 3.5 kg of goji berry fruit (cultivar: sweet berry) grown in an open field in Castellaneta (Taranto, Italy) was conventionally handpicked with a peduncle. Damaged fruits were removed, leaving 3.3 kg healthy fruit with homologous dimensions (major axes, $11.82 \text{ mm} \pm 1.24 \text{ mm}$; minor axes, $9.4 \text{ mm} \pm 0.69 \text{ mm}$). Approximately 120 g of fruit was used for the initial evaluation; then, the fruits were split into 27 groups (approximately 117.8 g each)—three replicates for storage at 0,

5, and 7 °C under 95 % of relative humidity (RH). Quality attributes of goji berry fruit were determined on the day of harvest and after 5, 9, and 12 days of storage.

2.2. Physical Quality Attributes

The color of the goji berry fruit was measured by extracting images acquired with a spectral scanner (DV s.r.l., Italy); color parameters were quantified in the CIE L*a*b* color space. Hue° = arctg $\frac{b^*}{a^*}$ and chromaticity = $\sqrt{a^{*2} + b^{*2}}$ were calculated from the a* and b* values (Danial Fatchurrahman, Amodio, de Chiara, Chaudhry, & Colelli, 2020).

Firmness was assessed on 20 fruits for each replicate by applying a compression test to rupture the fruit between two parallel plates using a texture analyzer (TA-XT2[®], Stable Microsystems, Godalming, UK) at a crosshead speed of 75 mm min⁻¹. The rupture load of the deformation curve was recorded in Newton (N).

2.3. Sensorial Analysis

The sensory evaluation of goji fruit was performed by four trained panel members using a method introduced by Miller et al. (2005) for apples, which was then applied to goji berries by Jatoi et al. (2018). The sensorial properties comprised firmness, texture, juiciness, sugar-acid ratio, aroma, tastefulness, and general impression. The external properties are shape, size, and color. The hedonic scale was ranked from 1 to 5 comprising excellent (5), very good (4), good (3), acceptable (2), and unsatisfied (1), where good indicates that the fruit is marketable, and acceptable means that fruit is edible for fresh consumption.

In addition, the incidence of mold and visual damage expressed as a percentage of the total number of fruits for each replicate, was recorded.

2.4. Electrolytic Leakage

Relative electrolyte leakage (REL) was evaluated based on the method reported by Navarro-Rico et al. (2015) with some modifications. Instead of water, mannitol isotonic solution was used to avoid osmotic shock (Saltveit, 2002); 0.4 mol L⁻¹ mannitol was determined to be the optimal concentration after following the procedure suggested by Peng et al. (2014). Approximately 10 fruit slices were taken from each replicate (approximately 3 g in weight) and placed in a centrifuge tube with 25 mL of 0.4 mol L⁻¹ mannitol (Sigma Aldrich, Steinheim, Germany). The electrical conductivity of the bathing mannitol solution was measured with a conductivity meter (CM35, Crison, Carpi, Italy) after 1 min (C1) and 60 min (C60) of incubation with orbital shaking (DAS12500, Intercontinental Equipment, Rome, Italy) at a speed of 60 cycles min⁻¹. The samples were then frozen at -20 °C for 24 h, and the conductivity (CT) was measured after defrosting for 3 h at 25 °C. The REL was calculated using the following equation:

$$REL (\%) = \left[\frac{(C60 - C1)}{CT} \right] \times 100$$

2.5. Physiological and Metabolic Attributes

The respiration rate (mg CO₂/kg/h) of goji berries was measured under static conditions following the protocol described by Kader (2002), with some modifications. Briefly, three replicates of 90 g fruit each were placed in 150-mL sealed glass jars with a plastic septum for sampling. The jars were initially left open in a temperature as well as humidity controlled room to acclimate the samples (0, 5, and 7 °C); after closing, gas samples (1 mL) were collected from each jar after the required time to accumulate CO₂ in the headspace up to a concentration of 0.1–0.2 % and injected into a gas chromatograph (Shimadzu, model 17 A, Kyoto, Japan) equipped with a thermal conductivity detector (200 °C). Separation of CO₂ was

achieved on a Carboxen 1006 plot (30 m × 0.53 mm, Supelco, Bellefonte, PA, USA) with a column flow of 7 mL min⁻¹ and an oven temperature of 180 °C. The respiration rate calculation was based on the differences in CO₂ concentration, sample weight, the free volume inside the jar, and elapsed time (Caleb et al., 2012). Ethylene production (μL C₂H₄/kg/h) was measured using the closed system introduced by Kader. (1992). The accumulation of C₂H₄ in the headspace of the sealed jars was measured using gas samples (2.5 mL) that were injected into a gas chromatograph (Agilent, model 7890 A) equipped with a flame ionization detector (FID). The detector temperature was set at 300 °C with a hydrogen flow of 45 mL min⁻¹ and airflow of 400 mL min⁻¹. For ethylene separation, a metal-packed column 13073-U (Supelco, Bellefonte, PA, USA) was used. Helium was used as the carrier gas (pressure, 15 psi). The oven temperature was set to 120 °C. The ethylene concentration was then referred to the sample weight, to the free volume in the jar, and to the elapsed time.

2.6. Maturity Index

A method introduced by Fatchurrahman et al. (2021) was used for measuring SSC, TA, and pH. Ten goji berries per replicate were homogenized in Ultra Turrax (IKA T18 basic, Germany) and then filtered with two layers of cheesecloth (JC NONSTE SWAB 4040, Shanghai, China). The obtained juice was used for direct SSC (%) reading using a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan). TA and pH were measured in 1 g of juice samples using an automatic titrator (T50 M Terminal, METTLER TOLEDO, Greifensee, Switzerland). The samples were titrated against a 0.1-mol L⁻¹ NaOH solution up to a final pH of 8.1; the results are reported as a percentage of citric acid per 100 g sample.

2.7. Chemical Composition

2.7.1. Determination of Ascorbic Acid, Dehydroascorbic Acid, and Vitamin C Content

Ascorbic acid (AA), dehydroascorbic acid (DHAA), and total vitamin C contents were assessed by homogenizing 5 g of fruit tissue in Ultra Turrax for 1 min with 5 mL of methanol/water (5:95 v/v), citric acid (21 g L⁻¹), ethylenediaminetetraacetic acid solution (EDTA) (0.5 g L⁻¹), and sodium fluoride (NaF) (0.168 g L⁻¹). The homogenate was filtered through cheesecloth, and the pH was adjusted to 2.2–2.4 by the addition of 6 mol L⁻¹ Hydrochloric Acid HCl. The homogenate was centrifuged at 12.000 rpm for 5 min; then, the supernatant was recovered and filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and through a 0.2µm cellulose acetate filter. AA and DHAA contents were determined as described by Zapata and Dufour (1992) with some modifications. HPLC analysis was performed after the derivatization of DHAA into fluorophore 3-(1,2-dihydroxy ethyl) furol [3,4-b] quinoxaline-1-one (DFQ) with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 µL) were analyzed using HPLC system (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was methanol (MeOH)/ water (H₂O) (5:95 v/v), containing 5 mmol L⁻¹ cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate at pH 4.5. The flow rate was set at 1 mL min⁻¹. The detector wavelengths were 348 nm for DHAA and 251 nm for AA. The AA and DHAA contents are expressed as grams of AA or DHAA /kg f.w.

2.7.2. Determination of Anthocyanin Content

The anthocyanin content was determined using the method introduced by Proctor (1974). Two disks (top cut) were taken from fresh goji berries (approximately 1 mm thick). The area of the disks was calculated using the area of the ellipse formula $A = a \times b \times \pi$. Goji fruit disks were then mixed with 3 mL of acidified methanolic solution (10 mL HCl/L) until submerged and treated for 3 h at 25 °C under dark

conditions. The anthocyanin level was measured according to the formula introduced by Wells (1995):

$$Anthocyanin = Absorption_{532 nm} - 0.25 (Absorption_{653 nm})$$

Afterward, the molar concentrations of anthocyanins/cm² were obtained by dividing the optical density values by the molecular extinction coefficient of cyanidin (2.45 \times 10⁴) and then again dividing by the area of the leaf disks (Siegelman and Hendricks, 1957). The results are expressed as milligrams of cyanidin per cm².

2.7.3. Total Polyphenol Content and Antioxidant Activity

The total polyphenol content was determined using 5 g of goji berries homogenized in Ultra Turrax for 1 min in 30 mL medium containing 80 % methanol: 20 % water solution and 2 mmol L⁻¹ sodium fluoride. The homogenate was then centrifuged at 9000 rpm for 10 min at 4 °C. The method followed a protocol previously used by Derossi et al. (2016) with slight modifications. Briefly, 100 µL of the extract was mixed with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent, and 300 µL of sodium carbonate solution (200 g L⁻¹). The absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China) after allowing the solution to stand for 2 h. The total polyphenol content was calculated based on the calibration curve of gallic acid and expressed as milligrams of gallic acid per 100 g of fresh weight (mg GA 100 g⁻¹ f.w.). The antioxidant assay was performed according to the procedure described by Capotorto et al. (2018) with minor modifications. Fifty microliters of the same extract, opportunely diluted, was pipetted into 0.950 mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after 24 h of incubation. Trolox was used as a standard, and the antioxidant activity is presented as grams of Trolox equivalents per kg of fresh weight (g TE kg^{-1} f.w.).

3. Statistical Analysis

The effects of storage duration and storage temperature were analyzed using two-way ANOVA, and the significance of differences among means was determined using Tukey's test at P<0.05. All calculations were conducted using the statistical software IBM-SPSS 2019 (1 New Orchard Road, Armonk, New York 10504-1722, United States.

4. Results and Discussion

Quality attributes of goji berry fruit were determined at harvest day and days 5, 9, 10, and 12 of storage at 0, 5, and 7 °C. The effects of temperature, storage duration, and their interactions were analyzed using two-way ANOVA, and the results are shown in Table 1.

Table 1. Effect of storage temperature, storage duration, and interaction on quality attributes of goji berries stored for 12 days at 0, 5, and 7 °C. Mean values of 12 samples are reported (3 replicates × 4 storage durations).

Quality Attributes	Storage Temperature	Storage Duration	Storage Temperature × Storage Duration	
Weight loss (%)	****	****	****	
Hue (°)	**	**	ns	
Chroma	*	***	*	
Firmness (N)	***	ns	ns	
Mold damage (%)	****	****	****	
Shriveling damage (%)	****	****	****	
Black spot damage (%)	****	****	****	
REL (%)	****	*	*	
SSC (%)	****	****	****	
SSC/TA	*	*	ns	
TA (%)	*	ns	*	
AA (mg AA 100 g ⁻¹)	****	****	ns	
DHAA (mg DHAA 100 g ⁻¹)	****	ns	ns	
Vit. C (mg VitC 100 g ⁻¹)	*	****	ns	
Anthocyanin (mg cyanidin cm ⁻²)	****	****	*	
Total polyphenol (mg GA 100 g ⁻¹)	****	****	****	
Antioxidant (mg TE100 g ⁻¹)	****	****	****	
Firmness (sensory)	***	****	ns	
Texture (chewing; sensory)	****	****	ns	
Juiciness (sensory)	****	****	ns	
Sugar-acid ratio (sensory)	****	****	ns	
Aroma (sensory)	****	****	ns	
Tastefulness (sensory)	****	****	ns	
General impression (sensory)	****	****	ns	
Shape (sensory)	****	****	ns	
Size (sensory)	****	****	ns	
Color (sensory)	****	****	*	

Note: (****) $P \le 0.0001$; (***) $P \le 0.001$; (**) $P \le 0.01$; (*) $P \le 0.05$; ns, not significant.

Goji berry fruit underwent several types of damage during storage, depending on the temperature. Figure 1 presents the three types of damage found in goji berries during storage: shriveling, black spots, and molds.



Figure 1. External appearances of goji berry during storage: (A) sound, (B) shriveling, (C) black spot, (D) early spot of mold, (E) mold.

Both storage duration and storage temperature significantly affected shriveling and black spot and mold occurrence. Figure 2 depicts the incidence of damage to goji berries during storage. The manifestation of all damage types started after 5 days under the storage conditions, and the most severe mold infection was seen at 7 °C, reaching 22.91 % versus approximately 11 % at the lower temperatures (0 and 5 °C). As for shriveling, a higher incidence was observed in berries stored at 0 and 7 °C, both reaching 15 % and 5.34 %, respectively. The incidence of damaged fruit gradually increased over time, being the maximum 33.3 % of mold on day 12 of storage at 7 °C, as against approximately 20 % at 0 and 5 °C. Furthermore, the highest incidence of shriveling and black spots was found in goji berry fruit stored at 0 °C, where only 5.8 % of the fruit remained unaffected. Goji berry fruit stored at 5 °C showed the lowest incidence of shriveling and black spots, with a final percentage of sound fruit of 46.9 %, whereas fruit stored at 7 °C showed 21.1 % of sound fruit. Our results indicate that the storage temperature of 5 °C should be preferred over 0 °C; this is not in accordance with a study by Jatoi et al. (2018) that mentioned that storage at 0 °C best conserves the fresh goji berry fruit compared with when stored at -2 °C, 10 °C, and 20 °C.

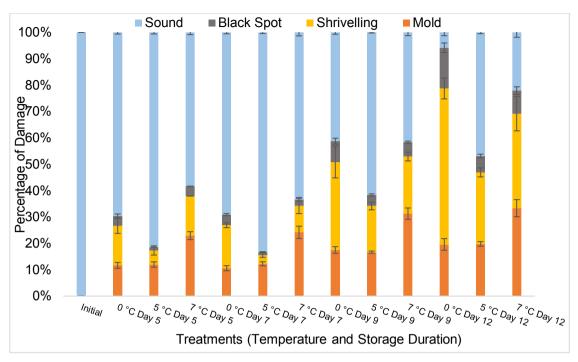
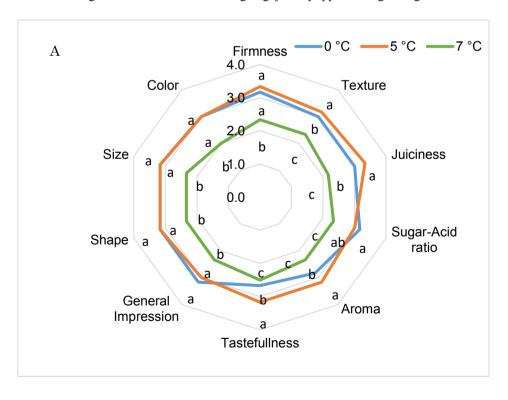


Figure 2. Distribution of damaged goji berry types during storage.



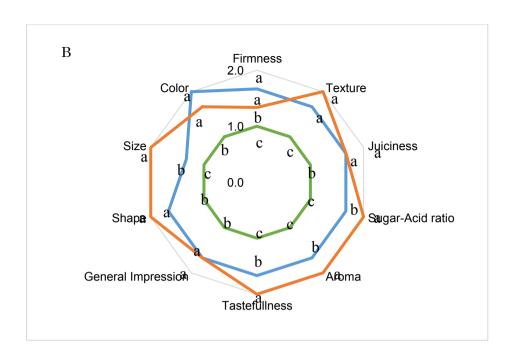


Figure 3. Sensory evaluation of goji berries stored at three different temperatures for 9 (A) and 12 (B) days.

The sensory attribute results revealed that goji berries could be stored with a good quality for about 9 days, although 16 % of the berries had very early infection of mold indicated by an early spot of mold on the surface of the fruit's skin (Figure 1D). As depicted in Figure 3, the fruit stored at 0 and 5 °C had a reasonable marketable acceptance with a hedonic score of 3 (good) for tastefulness and general impression. Furthermore, goji fruits stored at 5 °C were not significantly different from those stored at 0 °C but showed better performance compared with those stored at 7 °C, as the fruit had the highest hedonic scores for tastefulness, aroma, juiciness, texture, and firmness (all scores, > 3). In contrast for fruit stored at 7 °C, the scores were between 2 and 2.5. Although the sensorial attributes of fruits at 0 °C were acceptable, fruits stored at 5 °C were of the best quality (Figure 3).

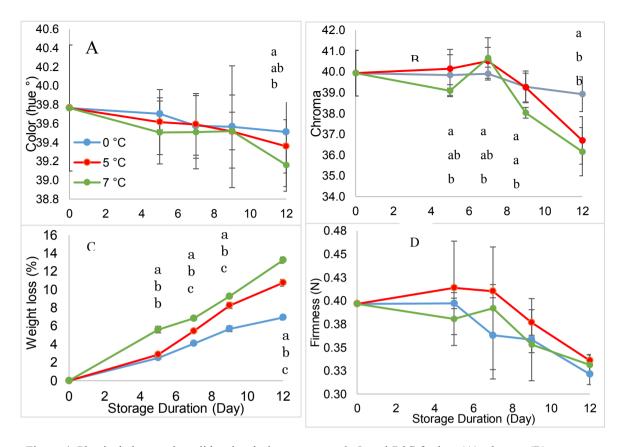


Figure 4. Physical changes in goji berries during storage at 0, 5, and 7 °C for hue (A), chroma (B), weight loss (C), and firmness (D). Values marked with the same letter on the same harvest day are not significantly different, according to Tukey's test.

Color is an important parameter for determining the quality of produce; carotenoids give a bright red-orange color in fresh goji berries (Q. Zhang et al., 2016). Jatoi et al. (2017) reported that fresh mature goji berries contain as much as 321.09 μg.g⁻¹ β-carotene. Additionally, among β-carotene types, the content of zeaxanthin esters in goji fruit can exceed 77.5 % of βCE/g f.w., and zeaxanthin palmitate is especially abundant, reaching 31–56 % of mg βCE/g f.w. (Y. Peng et al., 2005). As shown in Figure 4A, during storage, goji berry fruit did not show any significant difference until day 12, whereas fruit at 7 °C showed a lower hue value than fruit stored at 0 °C because of the faster senescence and high incidence of rotten fruit. This contrasts the findings of Jatoi et al. (2018), who did not report any difference in color during storage of goji berries for 12 days between -2 and 20 °C. The effect of temperature

on accelerating senescence and degradation in terms of color and other attributes is very predictable (Kasmire & Kader, 1978). Fruits stored at 0 and 5 °C maintained the best color quality.

As the storage duration increased during the experiment, the fruit lost water and weight, as shown in Figure 4C. A significant weight reduction was expected due to the berry's highly perishable nature; however, the rate of weight loss was greatly influenced by storage temperature. Fruit stored at 0 °C showed the lowest weight loss (7 %) on day 12; these values were significantly different from the weight loss observed for samples stored at 5 °C (11 %) and 7 °C (13 %). Jatoi et al. (2018) reported that goji berries showed higher weight loss (18 %) when stored at 10 °C than when stored at 0 °C (13 %); the latter storage temperature was better for retaining fruit freshness up to 12 days of shelf-life. During storage, the fruit firmness also decreased. Fruit softening is caused by cell wall hydrolysis, where more than 50 genes related to cell wall structure show variation in expression, involving complex quantitative trait loci (Seymour, Chapman, Chew, & Rose, 2013).

The fruit respiration rate increases with storage temperature (Kader, 2002). The berries stored at 7 °C attained the highest respiration rate at 28.24 CO₂/kg/h, while the rates at 5 °C and 0 °C were 13.33 and 5.82 CO₂/kg/h, respectively (Table 2). In addition to the effect of temperature on fruit metabolism, the higher respiration values in fruit stored at 7 °C may result from increased cell damage or microbial growth (Rodoni et al., 2014). The rate of ethylene production of goji berry fruit was at a moderate level when stored at 7 °C (3.83 μL C₂H₄/kg*h) and at low levels at 0 and 5 °C (0.67 and 0.89 μL C₂H₄/kg*h, respectively). Zhou et al. (2020) reported a respiration rate and ethylene production of 80 mL CO₂/kg/h and 30 μL C₂H₄/kg/h, respectively, for goji berries stored at 4 °C; we observed a lower metabolic activity in comparison to these findings. This difference may be attributed to the different cultivar of goji berry used by Zhou et al. ('Zhongkelvchuan'). Our result is in line with that presented in another study where the respiration rate of goji berry at 7 °C

was 27.1 CO₂/kg/h (Palumbo et al., 2020).

Table 2. Respiration rate and ethylene production in goji berries at different

temperatures.

Temperature	Respiration rate	Standard Error (SE)	
	mL CO ₂ /kg/h		
7 °C	28.240	3.462	
5 °C	13.324	0.847	
0 °C	5.821	0.827	
Temperature	Ethylene production	Standard Error (SE)	
•	μL C2H4/kg*h	, ,	
7 °C	3.832	0.109	
5°C	0.889	0.165	
0 °C	0.667	0.003	

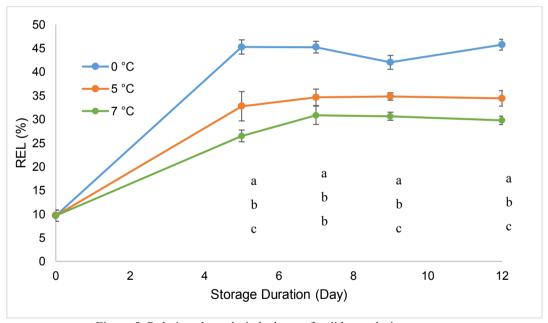


Figure 5. Relative electrolytic leakage of goji berry during storage. Values marked with the same letter on the same harvest day are not significantly different, according to Tukey's test.

Figure 5 depicts the electrolytic leakage of the goji berries during storage. Temperature significantly affected the electrolytic leakage level in goji berries. Fruit stored at 0 °C showed the highest electrolytic leakage of 46 % after 12 days, whereas berries stored at 7 °C showed the lowest value (30 % toward the end of the storage

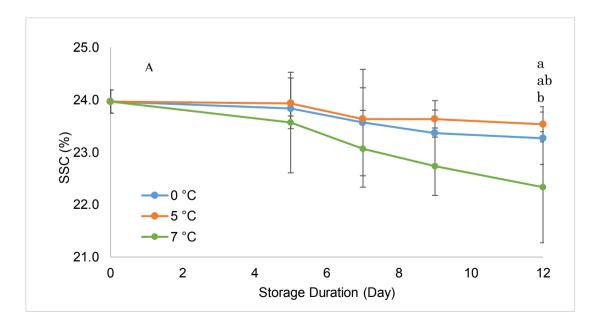
period). Thus, both temperature and time caused integrity loss and increased permeability. This is also explained by the increased plasma membrane permeability at 0 °C in eggplant (Babellahi, Amodio, et al., 2020). Furthermore, the electrolytic leakage of goji berries increased during storage; this was possibly caused by the loss of membrane integrity of the fruit in response to environmental stresses. Electrolytes are contained within membrane-bound compartments in living cells. The proteins and lipids of these membranes are degraded and oxidized under stress, leading to structural changes that cause loss of integrity and increased membrane permeability (Campos, Quartin, Ramalho, & Nunes, 2003).

Chemical and Nutritional Aspects

Table 3. Chemical and nutritional quality of goji berries stored up to 12 days at 0, 5, and 7 °C. Values marked with the same letter on the same harvest day are not significantly different according to Tukey's test.

		SSC/TA	AA (g kg-1)	DHAA (g kg-¹)	TP (g gallic acid kg ⁻¹)	AoxA (g Trolox kg¹)	AC (mg cyanidin-3- glucoside cm ⁻²)
Day	0	34.61 ± 1.449	0.254 ± 0.023	0.155 ± 0.015	2.995 ± 0.051	2.157 ± 0.087	1.185 ± 0.03
Day 5	0 °C	39.27 ± 0.45	$0.221 \pm 0.01a$	0.075 ± 0.006	$2.592 \pm 0.014b$	$1.834 \pm 0.039b$	1.263 ± 0.389
	5 °C	40.59 ± 1.11	$0.18 \pm 0.01b$	0.111 ± 0.043	$2.693 \pm 0.071a$	$1.897 \pm 0.025b$	0.978 ± 0.412
	7 °C	40.59 ± 3.67	$0.115\pm0.01c$	0.147 ± 0.029	$2.716 \pm 0.009a$	$2.269\pm0.08a$	1.286 ± 0.441
	0 °C	39.73 ± 1.59	$0.193 \pm 0.02a$	$0.067 \pm 0.036b$	$2.352 \pm 0.033b$	2.106 ± 0.021b	0.853 ± 0.124
Day 7	5 °C	39.76 ± 1.98	$0.14\pm0.02b$	$0.105 \pm 0.012ab$	$2.750 \pm 0.033a$	$2.322 \pm 0.035a$	0.813 ± 0.342
	7 °C	39.77 ± 2.39	$0.076\pm0.02c$	$0.134 \pm 0.004a$	$2.395 \pm 0.062b$	$2.336 \pm 0.059a$	1.098 ± 0.317
Day 9	0 °C	43.15 ± 1.68a	$0.122 \pm 0.032a$	$0.049 \pm 0.008a$	2.711 ± 0.063a	2.106 ± 0.07	0.983 ± 0.203
	5 °C	$38.49 \pm 1.76b$	$0.091 \pm 0.023 ab$	$0.112 \pm 0.007b$	$2.374 \pm 0.071b$	2.322 ± 0.028	0.857 ± 0.326
	7 °C	$38.33 \pm 1.59b$	$0.052 \pm 0.019b$	$0.133 \pm 0.004c$	$2.258 \pm 0.029b$	2.336 ± 0.092	1.159 ± 0.093
Day 12	0 °C	41.63 ± 0.76a	$0.091 \pm 0.01a$	$0.073 \pm 0.013b$	$2.545 \pm 0.083a$	$2.746 \pm 0.054b$	1.078 ± 0.523
	5 °C	$38.20\pm0.87b$	$0.049 \pm 0.01b$	$0.125 \pm 0.017a$	$2.224 \pm 0.067b$	$2.971 \pm 0.064ab$	1.361 ± 1.028
	7 °C	$36.17 \pm 1.46b$	$0.02\pm0.01c$	$0.122 \pm 0.007a$	$2.126 \pm 0.054b$	$3.216 \pm 0.082a$	0.917 ± 0.168

Table 3 shows the overall changes in the goji berry chemical composition during storage at different temperatures. There were no significant differences in terms of anthocyanin content, but SSC, TA, the SSC/TA ratio, and other nutritional components (AA, DHAA, vitamin C, and total polyphenol content) and antioxidant activity varied significantly. Figure 6 shows the SSC of the goji berries during storage. Soluble solids decreased with storage duration; the decrease at 7 °C was higher, reaching 22.33 % versus 23 % and 23.5 % at 0 and 5 °C, respectively, at the end of the storage period. These results are in accordance with the findings obtained by Ban et al. (2015), who found a decrease in total soluble content from 21.68 % (initial) to 17.32 % (after storage) in the control samples of Chinese wolfberry fruit stored at 2 ± 0.5 °C. Another study indicated climacteric behavior in goji berries (Fatchurrhamann, in press); therefore, an increase in SSC may be expected. Nonetheless, the maximum SSC reported for the more advanced maturity stage in this study was 23.5 %, which remained constant over the 12-day storage period. The present study results confirm that fruits harvested at maturity are ripe and already in the senescent phase. Low storage temperatures delay senescence, keeping SSC almost unchanged.



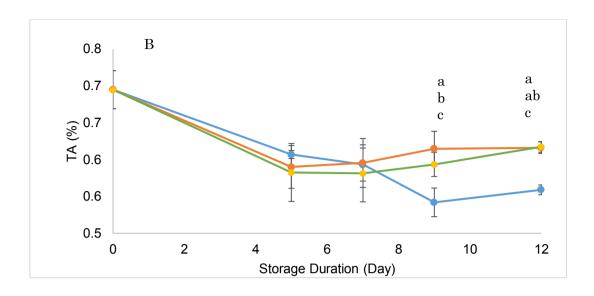


Figure 6. Change in soluble solid content (SSC) (A) and titratable acidity (TA) (B) during storage.

The TA of goji berry fruit changed during storage; it was 0.69 % on harvest day and decreased to ~0.56 % at 0 °C and 0.62 % at 5 and 7 °C of storage. Such changes, particularly evident at 5 and 7 °C, could be due to citric acid reduction during the ripening process (Ban et al., 2015), as observed in blueberries (Forney, W, MA, MR, & SAE, 2008). The significantly higher decrease in TA at 0 °C may be caused by the loss of organic acids and minerals because of chilling injury, as also seen in tomatoes (Ibrahim, Rhani, & Buhri, 2013). In addition, the higher percentage of water loss observed at 5 and 7 °C could be the reason for the concentration of higher TA compared to 0 °C, resulting in a slower reduction, as observed for SSC in raspberry fruit (Robbins et al., 1989). These results were reflected in the SSC/TA ratio trend, which at 12 days was higher for fruits stored at 0 °C (41.63) compared with that of fruits stored at 5 and 7 °C (38.2 and 36.17, respectively).

Goji berries are rich in vitamin C, which is beneficial for human health (González-Molina et al., 2010). Figure 7 depicts the vitamin C level (AA + DHAA) in goji berry fruit during storage. Vitamin C levels decreased during storage for 12 days under all storage temperatures. The level was decreased from 0.408 to 0.142 g/kg

f.w. for fruit stored at 7 °C, which was significantly lower than that for the sample stored at 5 °C (0.175 g/kg f.w.), while samples stored at 5 °C showed an intermediate content (0.163 g/kg f.w.). Goji berries stored at 7 °C showed the lowest vitamin C levels, probably because of the high levels of oxidation occurring at higher temperatures, as indicated by the higher value of the DHAA content (Mastrandrea, Amodio, de Chiara, Pati, & Colelli, 2017). The same trend with a slightly higher content at 5 °C compared to that at 0 °C and a reduction at higher temperatures have been observed in purslane leaves (Rinaldi et al., 2010). AA is a known antioxidant, and it is oxidized to DHAA in many reactions involving polyphenol oxidase, cytochrome oxidase, and peroxidase. The higher incidence of DHAA versus AA forms, found at higher temperatures, and the higher vitamin C reduction observed at 0 °C compared to that at 5 °C may be explained by an intense oxidase activity, as described previously (Gil-Izquierdo, Gil, Conesa, & Ferreres, 2001), which at 0 °C may be due to a chilling injury.

Our result is in accordance with that presented by Donno et al. (2015); they reported that goji berries contain approximately 40 mg/100 g f.w. of vitamin C on the day of harvest, which is typical of citrus fruits. Therefore, goji berry has the highest vitamin C content among such fruits but should be stored in the proper conditions since the content is halved after 7 days of storage.

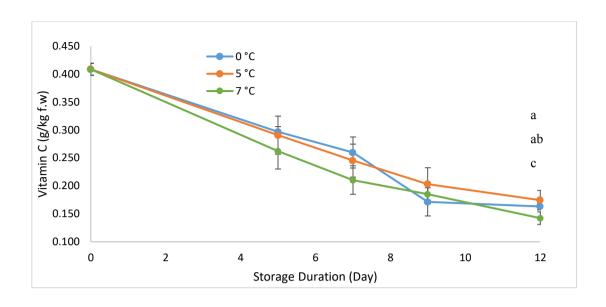


Figure 7. Changes in vitamin C levels during storage.

Values marked with the same letter on the same harvest day are not significantly different according to Tukey's test.

Significant differences were observed in the total polyphenol content in goji berries stored at 0, 5, and 7 °C (2.55, 2.25, and 2.126 g/kg, respectively) after 12 days (Table 3). The higher level of total polyphenol at 0 °C was maybe due to the oxidation of the phenolic compounds in the fruit stored at higher storage temperatures of 5 and 7 °C (Réblová, 2012). The range and abundance of phenolic compounds in fruit may vary depending on geographical location, genetic variation, region, agricultural practices, cultivation method, year of harvest, growth period, or storage conditions (Dong et al., 2012). Donno et al. (2015) reported that the content of polyphenolic compounds in Lycium spp. was 9.41 g/kg f.w., whereas Wang et al. (2010) reported this value to be 1.42 g/kg. The DPPH antioxidant activity was significantly lower in fruits stored at 0 °C (2.74 g Trolox/kg) than in those stored at 5 °C (2.97 g Trolox/kg) and 7 °C (3.216 g Trolox/kg) on day 12 (Table 2). This may be due to the contribution of the secondary metabolites zeaxanthin and β-carotene—abundant in goji berry fruit that is best conserved at temperatures 7–10 °C (Kulczyński & Gramza-Michałowska, 2016). This result is in accordance with that presented by (Jatoi et al., 2018), though goji berry stored at 10 °C for 12 days showed a higher DPPH antioxidant activity level (2.8 g Trolox/kg) compared with that in fruits stored at 0 °C (2.3 g Trolox/kg). Furthermore, a significant difference in DPPH activity was observed based on the day of storage, as the level of antioxidant activity increased from the initial day.

5. Conclusions

Storage temperature is a key factor for the proper storage and handling of fruits. This study complements existing information on the effect of low storage temperature on goji fruit quality and storability. If the temperature of 0 °C was confirmed to best maintain the quality of goji fruit in comparison to 7 °C, the results indicated that 5 °C should be recommended as optimal storage temperature, since it induced the lowest level of physiological disorders, while preserving physical, sensorial and nutritional quality attributes. A moderate chilling sensitivity was in fact observed at 0 °C, in the form of black spots, shriveling, and a higher incidence of decay. The findings indicated that goji fruit may be stored for about 9 days at 5 °C, but additional technologies such as modified atmosphere packaging may be needed to better control decay and to allow a safe distribution and consumption.

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Author Contribution

DF Conceptualization, Methodology, Software, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

MLA: Conceptualization, Methodology, Data curation, Supervision, Writing -

review & editing.

MLVDC: Formal analysis of Vitamin C, data curation, review and editing

MN: Methodology, Software, Data curation

LM: Formal analysis of Vitamin C;

GC: Conceptualization, Supervision, Writing - review & editing.

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Effect of Controlled Atmosphere with High Carbon Dioxide on Quality of Goji Berry Fruits (*Lycium barbarum* L.)

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Abstract:

Goji berries (*Lycium barbarum* L.) are functional fruits with high antioxidant values, which are usually marketed as a dried or processed product, because of their short shelf life. The aim of this study was to investigate the response of fresh goji fruits to carbon dioxide (CO₂). Sample replicates of about 50 g were stored in air enriched with different CO₂ concentrations (5 %, 10 %, 20 %, and 0 % for the control) for 22 days at 5 °C in humidified airflow. Firmness, overall appearance, soluble solid content (SSC), pH, total acidity (TA), texture, vitamin C, total phenol, antioxidant activity, and incidence of pitting and mold were evaluated over storage time. The results indicated that goji stored under a CO₂ concentration of 20 % showed the highest quality allowing a reasonable shelf life of up to 22 days. Fruits stored at 5 % and 10 % and in air showed higher susceptibility to decay represented as firmness losses and severity of mold infection. Regarding chemical composition, the main differences regarded antioxidant activity, which increased for the treatment at 5 % and 10 %. The information obtained is very important to design packaging for the distribution of fresh goji berries.

Key Words: packaging, antioxidant activities, shelf life, storage

1. Introduction

In recent years there has been an increasing demand for the consumption of wolfberry or goji berry (*Lycium barbarum* L.). This fruit belong to the family of *solanaceae*, well recognized worldwide due to its outstanding health benefit (Jatoi et al., 2018), often called 'berry of youth' due to its anti-aging properties (Yao et al., 2011). Originated from China spread from southeastern Asia to Europe in the 18th century (Zhang et al., 2021; Kulczyński and Gramza-Michałowska, 2016). Similar to other berry types, mature fresh goji berry possesses tender and juicy tissue and is very susceptible to damage and microbial rot after harvest. Commonly, the

consumption of goji berries is cooked, processed as tea, soups, or served with meat and vegetables. It is also consumed in dried form (Zhu & Zi, 1998). The fruits are also utilized for the production of juice, tincture, and wine (Amagase and Farnsworth, 2011; Benzie and Wachtel-Galor, 2011; Potterat, 2010).

Dried goji berry is preferable for storage and marketing purposes, but the drying

process can cause the loss of nutritional properties of the fruits. With the growth of consumer's consciousness about nutrition and health, fresh fruit consumption is becoming preferable, as fresh goji berry fruits also have a pleasant taste. However, due to the fruit's perishability and susceptibility to damage and handling as well as distribution are difficult (Fan et al., 2019). For this reason, fresh wolfberry fruits are only available in areas where they are cultivated. Therefore, to promote the consumption and distribution of fresh goji berry, postharvest treatments to prolong the shelf-life of goji berry have become a big challenge for researchers worldwide. A recent study demonstrated that goji is a climacteric fruit (Fatchurrahman et al., 2022 submitted) and that can be stored for about 9 days at 5 °C, while evidence of chilling injury was reported for 0 °C (Fatchurrahman et al, submitted). Shelf-life is limited by the high susceptibility to decay and shriveling. Among the most important postharvest factors to be considered for fruit storage and distribution, the atmosphere composition plays a very important role, after humidity and temperature, and needs important consideration for packaging design. While several studies evaluated different postharvest treatments as dipping (Jatoi et al., 2017b) or edible coating (Fan et al., 2019), combined or not with mild heat treatment (Ban et al.; 2015), few studies exploited the effect of gas composition on quality of goji berries. The use of Passive Modified Atmosphere Packaging (MAP), reaching at equilibrium approximately 10 % of CO₂ prevented weight losses and organoleptic quality losses while avoiding molds occurrence up to 13 days at 7 °C (Palumbo et al. 2020). Additionally, Kafkaletou et al. (2017) tested short term CO₂-enriched atmospheres for 2 days before storage in air concluding that treatments of gas mixtures of Oxygen and CO_2 (5 % + 15 %) and (20 % + 20 %), can maintain the quality of stored goji

berries up to 14 days. The authors reported the effect of short-term treatments with high CO₂ and low O₂ concentration (i.e. 21% O2 + 0 % CO2, 5 % O2 + 15 % CO2, 10 % O2 + 10 % CO2 and 20 % O2 + 20 % CO2 (all balanced with N2) on the shelf-life of fresh goji berries during cold storage at 1 °C. This report however is limited by the fact that the authors reported only a short-term treatment (i.e. 2 days), in a static condition (fruits were placed in a polyethylene bag injected with a mixture of desired gas and without any further control of the gas concentrations). Furthermore, to the best of our knowledge, the effect of different CO₂, concentrations on the quality and storability of goji berry is not well exploited. CO₂ concentration as high as 20% if tolerated by the fruit, can prevent decay as reported for strawberries (Colelli & Martelli, 1995) and raspberries (Kader, 2003). For this reason, controlled atmosphere storage with different levels of carbon dioxide were applied during storage at 5 °C of goji berry fruits.

2. Materials and Method

2.1. Sample Preparation

The total amount of 3.6 kg goji berry fruit (*Lycium barbarum* L.; Cultivar: sweet berry) grown in an open field in Castellaneta (Taranto, Italy) conventionally handpicked with fruit peduncle were transpoted within 3 hours from harvest to the Postharvest laboratory of University of Foggia. Damaged fruits were removed leaving 3.3 kg healthy fruits with homogenous dimensions. As many as 300 g fruits were used for the initial evaluation, then the remaining 3 kg fruits were split into 12 groups (4 treatmentsX3 replications) for the controlled atmosphere storage treatment with a continuous flow of CO₂ concentration of 5 %, 10 %, and 20 % (balanced with nitrogen) and air as control. Each atmosphere was flushed to 3 different containers considered as a replicate consisting of about 250 g goji berry fruit with the flow speed set at 150 mL/min. All groups were stored at 5 °C under 95 % relative humidity (RH) for 22 days. The sensory evaluation, firmness, vitamin C, total phenolic compounds, and antioxidant activity were conducted on harvest

day, days 6, 11, 15, and after 22 days of storage.

2.2. Sensorial Analysis

The sensory quality of goji berry fruit was evaluated by four trained panel members by using a method introduced by Miller et al. (2005), defining the sensorial properties as firmness, texture, juiciness, sugar-acid ratio, aroma, taste-fullness, and general impression whereas external properties were expressed as shape, size, and color. The hedonic scale was ranked from 1-5 comprising excellent (5), very good to excellent (4.5), very good (4), good to very good (3.5), good (3), average (2.5), acceptable (2), unsatisfied to acceptable (1.5) and unsatisfied (1). The number of berries infected by molds and visual damage was expressed as a percentage.

2.3. Soluble Solid Content, Titratable Acidity, and pH

For the measurement of soluble solid content (SSC), titratable acidity (TA) and pH, 5 g of goji berries were homogenized in an UltraTurrax (IKA T18 basic, Germany), then filtered with two layers of cheesecloth (JC NONSTE SWAB 4040, China) and the obtained juices were employed for direct reading of the SSC (%) using a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan). TA and pH were measured using 1 g samples of the juices using an automatic titrator (T50 M Terminal, METTLER TOLEDO, Switzerland). The samples were titrated against a 0.1 mol L⁻¹ NaOH solution up to a final pH of 8.1 and were reported as a percentage of citric acid per kg sample.

2.4. Determination of Ascorbic Acid, Dehydroascorbic Acid, and Vitamin C

Ascorbic acid, dehydroascorbic acid, and total vitamin C amounts were assessed homogenizing 5 g fruit tissue in an Ultraturrax for 1 min with 5 ml of methanol/water (5:95 v/v), plus citric acid (21 g L^{-1}), EDTA (0.5 g L^{-1}), NaF (0.168 g L^{-1}). The homogenate was filtered and treated as described by Derossi et al. 2016. AA and

DHAA contents were expressed as a gram of ascorbic or dehydroascorbic acid per kg of fresh weight.

2.5. Total Polyphenol and Antioxidant Activity

The same extract was used for total phenol content and antioxidant activity, homogenizing 2.5 g of goji berries with an Ultraturrax for 1 min in 15 mL medium of 80 % methanol:20 % water solution 2 mmol L⁻¹ in sodium fluoride. The homogenate was then centrifuged at 9000 rpm for 10 min at 4 °C. The method used was following a protocol previously used by Singleton and Rossi (1965). 100 µL of extract were mixed with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent, and 300 μL of sodium carbonate solution (200 g L⁻¹). The absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China) after t 2 h. The content of total phenols was calculated based on the calibration curve of gallic acid and expressed as milligrams of gallic acid per 100 g of fresh weight (mg GA 100 g⁻¹). The antioxidant assay was performed following the procedure described by Brand-Williams et al. (1995) with minor modifications. Fifty microliters of extract, opportunely diluted were pipetted into 0.950 mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after 24 h incubation. Trolox was used as a standard and the antioxidant activity was reported in a gram of Trolox equivalents per kg of fresh weight (g TE kg⁻¹).

2.6. Statistical Analysis

The significance of differences among means was obtained using the Tuckey test at p < 0.05. All the calculation was conducted by using the statistical software IBM-SPSS 2019.

3. Result and Discussion

The effect of CA on the quality of goji berry was assessed and the quality attributes

of goji berry fruits were determined during 22 days of storage. Figure 1 depicts the physical appearance of goji berry fruits stored under different CA treatments. Physical quality was compromised by the occurrence of 2 kinds of defects, named mold and visual damage, calculated as a percentage. Visual damage was indicated by the wrinkled surface of the skin and followed by the occurrence of black spots, that later turned in mold development.



Figure 1. External appearances of goji berry after 22 days of storage: (A) Control, (B) 5% CO₂, (C) 10 % CO₂, (D) 20 % CO₂.

As can be seen in figure 1, CA with 10 % of CO₂ and CA with 20 % CO₂ better controlled the occurrence of decay than air and CA with lower CO₂ concentration. The incidence (%) of mold and visual damage is shown in figure 2. It can be seen that after 22 days the treatment of 20 % CO₂ showed a minimum level of mold and visual damage (3 and 9%) while samples stored in air and air with 5 % CO₂ were completely spoiled. Fruit under 10 % CO₂ showed the occurrence of mold and visual damage at 12 % and 17 % respectively. However, fruits treated with 5 % CO₂ and

control in the air already deteriorated at day 15 so after this day, chemicals and physical evaluation were not assessed, and all sensorial attributes were scored 1 for the control fruit, being considered as inedible. This result is in accordance with the previous studies of the effect of CO₂ to prevent decay in several commodities. High CO_2 treatment with a concentration from 10 - 30 %, can significantly inhibit the development of brown rot disease from fungus Monilial fructicola on inoculated sweet cherry, the development of brown rot symptoms was not discovered on the fruit, allowing the longer shelf-life of up to 30 days at 0°C compared to control (air treatment) which fruit deteriorated after 4 days of storage (Tian, Fan, Xu, Wang, & Jiang, 2001). A similar result was found on wild strawberry fruit that combination treatment of 10 % CO2 and 11 % O2 at 3 °C can prolong the shelf life of wild strawberries, inhibiting the development of *Botrvtis cinerea*, and allowing to double the shelf-life from 10 days in air up to 20 days (Almenar, Hernández-Muñoz, Lagarón, Catalá, & Gavara, 2006). CO2 treatment also improves tolerance to prolonged cold storage in some commodities. Ezz et al. (2004) reported that treatment with high levels of CO₂ reduced peel pitting in grapefruit by delaying proline metabolism. CO2 treatment before storage at 2 °C effectively reduced physiological decay in zucchini (Serrano, Pretel, Martínez-Madrid, Romojaro, & Riquelme, 1998). However, to the best of our knowledge, there is not any report on the use of continued CA for the conservation of goji berry fruits. The only report available refers to the use of short CO₂ treatments (2 days) with different level of O₂ before the exposure to air for the rest of storage at 1°C, and show that goji berry fruits can reach a shelf-life of 14 days with the gas mixture of 15 % CO₂ and 5 % O₂ or combination of 20 % CO₂ and 20 % O₂ (Kafkaletou et al., 2017). Thus, allowing continued exposure to high CO₂ levels can sensibly increase the shelf-life of fruit up to 20 days, but it must be noticed that the temperature of storage of the present study is different, being 5 instead of 1 °C. This from one side confirms longer shelf-life despite the higher temperature of storage, but from the other side, having shown that goji is chilling sensitive (Fatchurrahman, submitted for publication), the lower shelflife at 1 °C can also be partially attributed to the improper temperature of storage. In this recent study on the effect of low temperature of storage, Fatchurrahmann et al. suggested 5 °C as the optimal temperature of storage between 0 and 7 °C, delaying excessive senescence, while preventing chilling injury. Goji berry stored at 5 °C showed the lowest percentage of mold and visual damage, which after 9 days were about 16%. Adding 20 % CO₂, therefore, resulted in a sensible extension of the shelf-life of goji which was more than doubled at the same temperature.

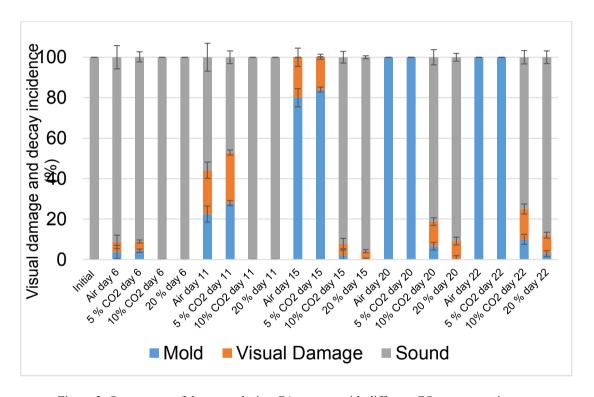


Figure 2. Occurrence of damages during CA storage with different CO₂ concentrations

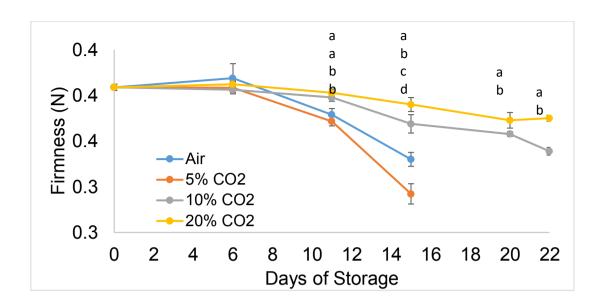


Figure 3. Firmness changes of goji berry fruits during CA storage with different CO₂ concentrations. Values marked with different letters at the same storage day are significantly different according to the Tuckey test.

Figure 3 depicts the firmness changes of goji berry fruits during storage in CA with different CO₂ concentrations. Firmness decreased over storage time, as expected. During storage, ripening progresses with fruit softening, color change from green to red, along with compositional changes in chemicals related to flavor and aroma, such as organic acids, sugars, and volatiles (Fatchurrahman et al., 2020; Fatchurrahman et al., 2021; Park et al., 2021). It can be observed that supporting the previous data on the occurrences of decay fruits stored under 5 % CO₂ showed the highest loss of firmness, being after 15 days was 0.33 N, followed by control in air (0.35 N) from 0.4 N if the initial day. Fruit stored in CA treatment with 20 % CO₂ almost retained the initial value, being higher than for fruit stored with 10% at 15 and 22 days of storage when its values was 0.38 N. The effect of high concentration of CO₂ exposure as pre-and postharvest treatment on firmness retention have been investigated in several commodities. Treatment of High CO₂ effectively maintained firmness in strawberries, allowing the longer shelf-life up to 10 days without altering the color, SSC, TA, and pH of the fruit (Bang, Lim, Yi, Lee, & Lee, 2019). Another

study on strawberries storage with 20 % CO2, reported an firmness increase up to 130 % after 4 days of storage which remained stable during 2 days (Larsen & Watkins, 1995).

Regarding the assessment of the sensory attributes, together with the incidence of damages and decay can be defined in 20 days, but generally, the fruit maintained after 22 days a reasonable marketable acceptance expressed by general impression with a hedonic scale of 3.2 (good to very good) and having all sensorial attributes of tastefulness, aroma, sugar-acid ratio, juiciness, texture, firmness, color, size and shape higher than 3 and within 3.5, whereas those stored under CA 10 % CO₂ had score values between at 2.4 and 2.7. Furthermore, fruit under CA 5 % and control were considered rotten with a hedonic scale for all attributes less than 1.5.

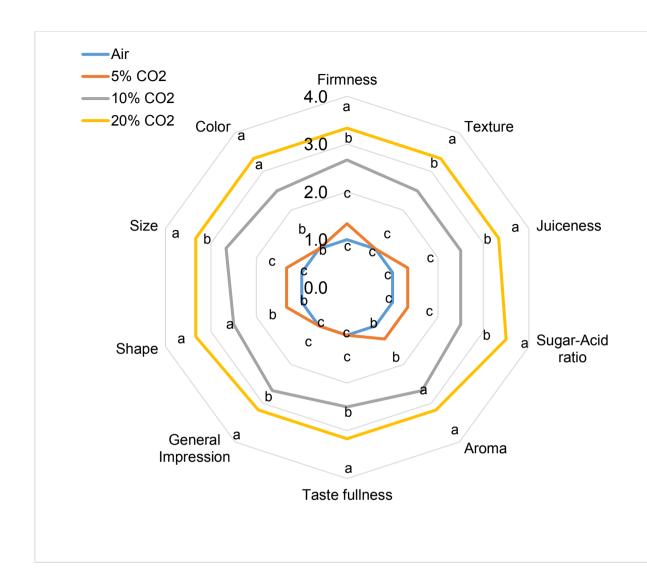


Figure 4. Sensory evaluation of goji fruits stored in CA under different carbon dioxide concentrations after 22 days. Values marked with the same letter on the same harvest day are not significantly different according to the Tuckey test.

Chemical and Nutritional Aspects

As for all the fruit, an important parameter of quality is the soluble solid content which represents the sweetness of the fruits. Goji fruit at harvest showed an SSC value of 22%, in line with the content of ripe fruit, as described by Fatchurrahman (2022, in press). It can be seen that fruits stored 20 % CA maintained the initial level

of SSC almost unchanged over 22 days of storage being significantly higher than the SSC value of fruit stored under CA 10 % which decreased to 18.3 % at the end of the storage. As for fruit stored in air s and CA with 5 % CO₂, a dramatic decrease in the SSC content was observed at the last sampling evaluation after 14 days of storage, when about 40% of the reduction was observed. These results showed that a decrease of SSC was observed for all treatments due to senescence and consumption of substrates for respiratory activity, which was delayed by the presence of high concentrations of CO₂ (Bang et al., 2019). Additionally, goji berry fruit stored under 20 % CO₂ maintained the highest SSC due to the fact that also damage and spoilage were very little, compared to those stored under 10 % and 5 % CO₂. The results however are in agreement with the findings obtained by Ban et al. (2015) as they found a decrease in total soluble in control samples of Chinese wolfberry fruits during storage which corresponds to the fruits manifestation of damages. Furthermore, regarding the titratable acidity of goji fruits at different CA treatments, we observed that TA of goji berry fruits showed a slight increase over storage from 0.5 % at harvest to 0.6 % after 22 in CA with 10 % and 20 % CO₂, and a sharper increase for fruit held in the air and CA with 5 % CO₂, after 6 days of storage. This is maybe due to ripening indicated by the increase of total acidity as reported by (Fatchurrahman et al. in press) that goji berry is climacteric fruit and will ripe after being detached from the plant followed by the senescence after 6 days of storage. As the CO2 delayed the ripening a sharper increase was observed for control fruit and in CA with 5 % CO₂.

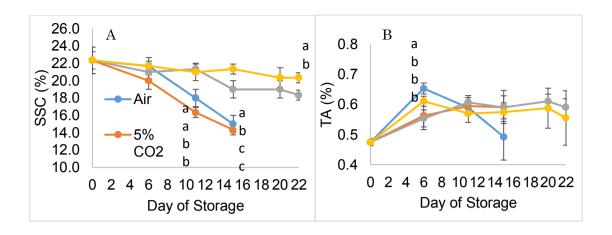


Figure 5. Change of SSC (A), TA (B), During Storage at three different CA treatments.

Values marked with the same letter on the same harvest day are not significantly different according to the Tuckey test.

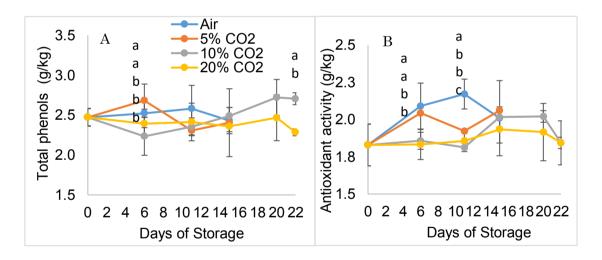


Figure 6. Change of total phenols (A), and total antioxidant (B), During Storage at three different CA treatments. Values marked with the same letter on the same harvest day are not significantly different according to the Tuckey test.

Figure 6. A depicts the concentration level of total phenols. We observed that the amount of total phenols remained enough stable during storage; however, a slight increase was observed after 5 days for samples stored in air and 5% CO2, while for

samples stored with 10 % CO2, phenols slowly increased from 5 to 22 days, reaching 2.71 g Gallic Acid/ kg. We may hypothesize that CO₂ inhibited phenols synthesis during postharvest storage (Kader, 2009), as also observed for grapes (Piazzolla, Amodio, Pati, & Colelli, 2021), and that for fruit stored with 10 % CO₂, this inhibition was less pronounced at the end of the storage. This trend was also observed for the total antioxidant activity of goji berry (Figure 6. B). The antioxidant content is similar to the previous literature reporting levels varying from 2-2.8 g Trolox/kg FW (Jatoi et al., 2017b) (Donno et al., 2015).

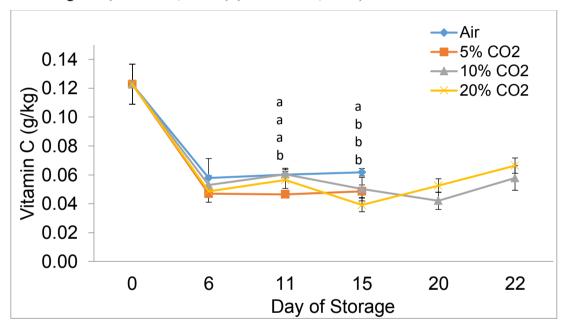


Figure 7. Change of vitamin C During Storage at three different CA treatments. Values marked with the same letter on the same harvest day are not significantly different according to the Tuckey test.

Figure 7 depicts the level of vitamin C of goji berry fruits during storage. We observed that vitamin C decreased during storage but the most important change for all treatments was observed already after 5 days of storage when Vitamin C was already more than halved. After 11 days of storage Vit C decreased from. 0.123 g/kg to 0.049 for samples stored with 5% CO2, showing a value significantly lower than

for the other treatments (being about 0.05 g/kg). The Vitamin C content remained almost stable up to the end of storage when no differences were observed for fruit kept in CA with 10 % and 20 % CO₂. This decrease may occur due to the ripening during storage. Compared to the previous report on vitamin level of goji berry from different genotypes revealed that our result gained a similar result that the vitamin C content varies between 0.12 to 0.19 g/kg (Kafkas et al., 2021). However, it is known that the differences between berries may also become the factors that affect vitamin C content, including cultural practice, maturity, climate, fresh fruit handling, processing factors, packaging, and storage conditions (Wojdyło, Nowicka, & Bąbelewski, 2018)

4. Conclusions

Maintaining the quality of the fruit and prolonging the storage life is the post-harvest purpose, and storage condition is the key factor of success. The fruits stored under 20% CA at 5 °C appeared to possess the highest fruit quality in terms of physical, sensorial, and phytochemical attributes with sound and fresh appearance after 22 days of storage compared to those of fruits stored under CA 5 %, 10 % and control which appeared with a higher level of damage and storage disorders. The findings obtained provide important information for the packaging design which providing 20% CO2 can ensure a shelf-life of about 20 days enabling its long-distance distribution and ensuring possible high standard quality

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Comparison Performance of Visible-NIR and Near-Infrared Hyperspectral Imaging for Prediction of Nutritional Quality of Goji Berry (Lycium barbarum L.)

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ABSTRACT

The potential of hyperspectral imaging for the prediction of the internal composition of goji berries was investigated. The prediction performances of models obtained in the Visible- Near Infrared (VIS-NIR) (400–1000 nm) and in the Near Infrared (NIR) (900–1700 nm) regions were compared. Analyzed constituents included Vitamin C, total antioxidant, phenols, anthocyanin, soluble solids content (SSC), and total acidity (TA). For vitamin C and AA, partial least square regression (PLSR) combined with different data pretreatments and wavelength selection resulted in a satisfactory prediction in the NIR region obtaining the R2pred value of 0.91. As for phenols, SSC, and TA, a better performance was obtained in the VIS-NIR region yielding the R2pred values of 0.62, 0.94, and 0.84, respectively. However, the prediction of total antioxidant and anthocyanin content did not give satisfactory results. Conclusively, hyperspectral imaging can be a useful tool for the prediction of the main constituents of the goji berry (*Lycium barbarum* L.).

Keywords: prediction; vitamin C; phenols; soluble solids; acidity

1. Introduction

Goji berry (*Lycium barbarum* L.) is widely recognized for its outstanding health benefit, a fruit of the family of Solanaceae (Jatoi et al., 2018). Originated from Asia and was introduced in Europe in the 18th century for its famous benefits for health and medical properties (Kulczyński & Gramza-Michałowska, 2016). Freshness is a quality attribute that determines the commercial values and sales of goji. A conventional method such as ultraviolet/visible spectrometry and HPLC can accurately determine the phytonutrients quality attributes such as antioxidant activity, total phenols, and multivitamins [3][4][5]). However, these analyses are time-consuming, need expensive instruments, and trained people, and as such can't

be used to assess the nutritional composition of individual fruit. Near-infrared spectroscopy has been utilized effectively to overcome these difficulties [6][7][8][9]). The hyperspectral imaging technique which is a combination of spectroscopic and imaging technique has been implemented due to its robustness of acquiring simultaneously the spectral and spatial information [6][7][8][10][11]. The Hyperspectral imaging technique requires a data analysis approach which is an essential step in phytonutrient quality determination. Recently, spectral preprocessing, wavelength selection and feature extraction, various modeling and model parameter optimization procedures have been used to improve the accuracy of the determination [8][12][13]. Calibration models are crucial for the determination of phytonutrients. High accuracy and robust models are preferable because of their high potential for industrial application. Partial least square (PLS) combined with interval partial least square (iPLS) for wavelength selections have been proven to be a robust method for the prediction of chemical compositions using near-infrared spectroscopy on apple [14]. The application of the PLS regression model has also been successfully used for the prediction of 6 different maturity stages of tomatoes from green to red by using a portable visible and near-infrared spectrophotometer (Huang, Lu, & Chen, 2018). Hyperspectral imaging allowed to discriminate the harvest time and to predict the internal content of soluble solids, phenols, and antioxidant activity of fennels (Amodio, Capotorto, Chaudhry, & Colelli, 2017), allowing to create of a concentration map for each component. Furthermore, recently in dried black goji berry, Zhang et al. 2020 [11] successfully predicted the total anthocyanin, total flavonoid, and total phenols by using the hyperspectral image method combined with PLS and LS-SVM, and [16] successfully predicted antioxidant activity combined with PLS regression model on dried black goji berry. However, available literature is lacking on research applications aimed to predict the nutritional content of fresh goji berry and therefore the objective of this study was comparing the performance of hyperspectral imaging method combined with PLSR in both region Vis-NIR and NIR to predict the

concentration of SSC, TA, vitamin C (dehydroascorbic acid plus ascorbic acid), anthocyanins, total phenols, and total antioxidant activity.

2. Materials and Methods

2.1. Sample Preparation and Spectral Acquisition

The total amount of 3.6 kilograms of goji berry fruit (*Lycium barbarum* L.; Cultivar: sweet berry) grown in an open field in the Province of Castellaneta (Italy) were harvested conventionally by picking the fruit with its peduncle. Four maturity stages of goji berry were harvested, starting from the early stage where fruit are still at the pinkish color with an average weight of 0.3 g, and average dimensions of approximately 9.89 mm and 7.23 mm for major and minor axis respectively, to the mature stage where fruit are at red color with an average weight of 1.3 g, possessing dimension of 16.26 mm and 13.15 mm respectively (figure 1).



Figure 1. Four maturity stages of goji berry (Stage 1 to 4 from left to right).

Damaged fruit were removed leaving 2.6 kilograms of sound fruit, after which fruit were scanned and classified based on the maturity stages resulting in a total of 383 images (92 images for vitamin C, AA, and DHAA, 97 images for total phenol and total antioxidants, 97 images for anthocyanin, 97 images for SSC and TA) the images were then split into 2 data sets for the prediction model analysis (70% were used for the calibration data set and around 30% for the prediction data set). Approximately 2.5 grams of homogenized fruit sample from around 5 fruit were

needed for individual chemical analysis, furthermore, as for the spectral analysis, a mean spectrum from those fruit was used.

2.2. Hyperspectral Image Acquisition

Hyperspectral image acquisition was done by using a hyperspectral line-scan scanner (Version 1.4, DV srl, Padova, Italy) consisting of two sensors, one in the visible near-infrared (Vis-NIR) region and the other in the near-infrared region (NIR). The region of VIS-NIR has a spatial resolution of 25000 × 12500 pixels/mm with a spectral resolution of 5 nm over a wavelength range of 400–1000 nm, however, in the NIR region, the spatial resolution was 7787.5 x 4000 pixels/mm and 5 nm spectral resolution covering the wavelength range of 900-1700 nm. In the case of Vis-NIR, a CCD camera was used, while a CMOS was for NIR with 50 frames per second equipped with C-mount lenses. A cooled halogen lamp with a stabilized power source was used as the excitation system. The GigE vision was used as the interface with a 37° field of view (FOV). Image thresholding, masking, and the extraction of the average spectra were done under MATLAB with a self-developed code.

2.3. Chemical Analysis, and Partial Least Square Regression (PLSR)

2.3.1. Determination of Vitamin C

Vitamin C, as the sum of Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents, was determined as described by (Zapata & Dufour, 1992) applying some modifications. Approximately 2,5 grams of fruit tissue in 5 ml of methanol/water (5:95), plus citric acid (21 g L⁻¹), EDTA (0.5 g L⁻¹), NaF (0.168 g L⁻¹) using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min. The homogenate was filtered by using cheesecloth, while the pH was adjusted to 2.2-2.4 by the addition of 6 mol L⁻¹ HCL. The analysis of HPLC was acquired after derivatization of DHA into the fluorophore 3-(1,2-dihydroxy ethyl) furol [3,4-b] quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA).

Samples of 20 µL were analyzed with an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump, as described in (Cefola et al., 2010). AA, DHAA and Vitamin C contents were expressed as g kg⁻¹ of fresh weight.

2.3.2. Determination of Anthocyanin

Total anthocyanin was determined by following a method introduced by [19]. Couples of discs (top cut) from fresh goji berries were taken (approx. 1 mm of thickness). The area was then calculated with an area of the ellipse formula $\mathbf{A} = \mathbf{a} \mathbf{x} \mathbf{b} \mathbf{x} \boldsymbol{\pi}$. Then, goji fruit discs were shaken in 3 ml of acidified methanolic solution (10 ml HCl/L) for 3 hours at room temperature in dark. Furthermore, the level of anthocyanin was determined based on the formula introduced by (Wells, 1995):

$Anthocyanin = Absorption_{532 nm} - 0.25 (Absorption_{653 nm})$

The molar concentrations of anthocyanins/cm² were acquired by dividing the optical density values by the molecular extinction coefficient of cyanidin (2.45 x 104), then divided by the area of the leaf discs. Hence, the results are expressed in mg of cyanidin per cm² (Jatoi et al., 2018).

2.3.3. Total Polyphenol and Antioxidant Activity

The determination of total phenol was done by using 2.5 g of goji berries homogenized in Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min in 80 % methanol: 20 % water solution 2 mmol L⁻¹ in sodium fluoride for 1 min. The resulted homogenate was then centrifuged under temperature 4 °C for 10 minutes at 9000 rpm. The method was done by following a protocol previously described by Cefola et al. (2010) (Cefola et al., 2010). The total phenols content was calculated based on the calibration curve of gallic acid per 100 g of fresh weight (mg GA 100 g⁻¹). The determination of antioxidant was done by a method introduced by [21] with few modifications (Cefola et al., 2010). Fifty microliters of diluted samples were mixed with 0.950 mL of DPPH solution to initiate the reaction. The

absorbance was measured at 515 nm after 24 h incubation. Trolox was used as a standard and the antioxidant activity was expressed in gram of Trolox equivalents per kg of fresh weight (TE g kg⁻¹).

2.3.4. Maturity indexes

The determination of soluble solid content (SSC), and titrable acidity (TA), was done by using 5 berries placed in a falcon tube, then homogenized in an Ultra-Turrax (IKA T18 basic, Germany), and filtered with two layers of cheesecloth (JC NONSTE SWAB 4040, China). The obtained juices were employed for direct reading of the SSC (%) using a digital refractometer (Atago N1, PR32-Palette, Tokyo, Japan), while 1 g juice samples were used for TA measurement by an automatic titrator (TitroMatic CRISON, Barcelona, Spain). The samples were titrated against a 0.1 mol L-1 NaOH solution up to a final pH of 8.1 and were expressed as a percentage of citric acid per 100 g sample.

2.3.5. Partial least squares regression (PLSR)

PLS algorithm for the desired parameters prediction models was developed by using PLS toolbox (Eigenvector Research Inc., version 7.2.5) working under MATLAB 2020b (version 9.9.0.1467703, MathWorks, MA, USA) as well as in HYPER-Tools (Version 3.0) HYPER-Tools works under Matlab environment and can be freely downloaded (https://www.hypertools.org/) (Mobaraki, 2018). The spectral dataset was divided into calibration set and validation set based on the 70/30 ratio with 70 % of the samples in the calibration dataset and 30 % of the samples reserved for external validation from the replicates of each acquisition interval. For the development of the PLSR calibration models, leave one out (LOO) cross-validation was applied. The optimum numbers of latent variables were chosen by using a convenient technique described by Haaland and Thomas, 1988 [23]. It consists of computing the ratios between the PRESS (Predicted Residual Error Sum of Squares) values and the minimum one. These PRESS ratios play the role of

variance ratios (analogous to the statistical F parameter) so that they can be associated with a probability p. The proposal, based on empirical results, is that the number of latent variables to be selected which the associated probability p is more than 0.75 (Olivieri, 2018). The accuracy of the calibration models was accessed by visualizing the root mean square error for calibration (RMSEC) and cross-validation (RMSECV). As the first approach different pre-treatments techniques by using all the wavelength were attempted including smoothing, mean centering, 1st and 2nd derivatization, and their combinations. Then, after the development of these models, the most significant variables were selected based on modified interval-PLS (Olivieri, 2018). In the modified interval-PLS method, the full spectral range was divided into sub-region of specific variables and then in each of these intervals, a separate model was formulated and evaluated by removing variables belong to the intervals. Finally, the eliminated intervals driving to improve accuracy were discarded from the full ranges.

Moreover, to detect the presence of outlying samples those whose nominal analyte concentration significantly deviates from the prediction when they are left out from the set, the following indicator, estimating the summation of deviations over the cross-validation process, was used (Haaland & Thomas, 1988):

$$F_{y}(i) = \frac{(I-1)(y_{pred,i} - y_{nom,i})^{2}}{\sum_{i \neq j} (y_{pred,j} - y_{nom,j})^{2}}$$

where $y_{pred,i}$, and $y_{nom,i}$ are the predicted and nominal value for the left-out sample during cross-validation, $y_{pred,j}$, and $y_{nom,j}$ are the corresponding values for the remaining samples, and I is the number of samples. The degrees of freedom for studying the significance of $F_y(i)$ are 1 and (I-1) for the numerator and denominator, respectively. Self-developed MATLAB code was used for modified interval-PLS and sampling to develop the PLSR approach.

All models were finally tested on the external data set to assess prediction performance. Moreover, for the best prediction models, the R² of calibration, cross validation, prediction, and root mean square error of prediction (RMSEP) were also assessed.

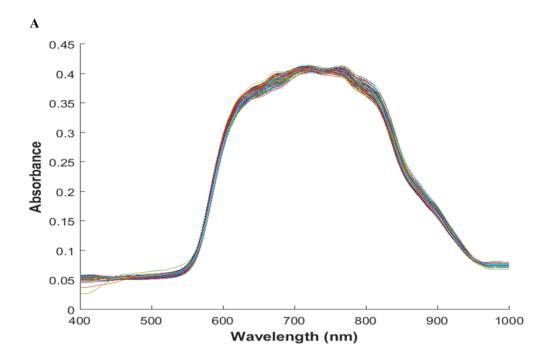
2.3.6. Mapping of internal constituents

Mapping of the internal constituent on the different stages of goji berry was done by firstly extracting an average spectrum from the pixels of the fruit image sample by considering that the mean spectrum corresponds to the average of a constituent of the fruit. So that, based on the PLSR models that were developed from the calibration dataset, the level of an internal constituent of goji berry was predicted in order to show the distribution of internal constituents of each goji fruit.

3. Results and Discussion

3.1. Spectral and Spatial Profile

Figure 2 shows the preprocessed reflectance spectra of goji berry in both regions of Visible Near-Infrared and Near-Infrared regions. However, the spectra obtained cannot be directly used for the determination of specific chemical constituents, since each spectrum reflects the complex constituent information. Data analysis approaches were performed to explore the relationship between spectra and vitamin C, AA, DHAA, total phenols, anthocyanin, SSC, and TA of goji berry.



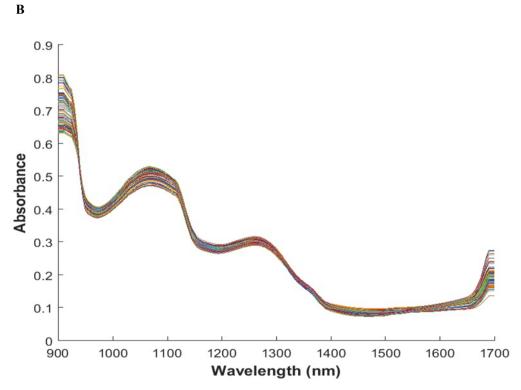


Figure 2. Pre-processed absorbance spectra of goji berry at the wavelength range of (A) VIS-NIR 400 nm -1000 nm and (B) NIR 900 nm - 1700 nm.

Figure 2 depicts the VIS-NIR and NIR spectra profile of goji berry. In the VIS-NIR peaks correlated to the color which are associated with phenols, carotenoids, anthocyanin, and chlorophyll compounds [25][26][27]. Additionally, some peaks found in the region of VIS-NIR at 900 nm - 970 nm are reported to overlapped peaks of starch, cellulose, sucrose, and water, and in particular, peaks from 900 nm to 920 nm are reported to correlate with starch and cellulose [28][29]. Regarding the NIR region, peaks generally represent the constituents of water and vitamin C. It has been reported that the peaks between 900 nm - 1000 nm and 1400 nm - 1500 nm are peaks corresponding to the constituent of water [30] [31]. Furthermore, peaks at 850 nm, 1000, 1210, 1360, 1460, 1580, 1650 nm have been reported to correlate with vitamin C in powdered mixtures and solutions (Yang & Irudayaraj, 2002), as also in spectra acquired with HIS on whole rocket leaves (Chaudhry et al., 2020).

3.2. Comparison of Prediction Model Between Spectra Range VIS-NIR and NIR

In Table 1 the mean values and respective range of composition for each nutritional quality parameter analyzed in this study are shown. Thanks to the different maturity stages, a large variation in the minimum and the maximum values for the chemical parameters were obtained to enlarge the interval of variation of the calibration models. The results obtained in this study are in a line with previous reports on the internal constituents of goji berry from different maturity stages as the vitamin C level increased as the fruits are more ripe and become softer followed by the increase in SSC and TA (Kafkas et al., 2021)(Donno et al., 2015). Regarding total phenol, antioxidant, and anthocyanin level of goji berry from different maturity stages, as the best of our knowledge there wasn't any report available yet, however our results on the ripe stage are in accordance with the previous reports (Jatoi et al., 2018)(Islam et al., 2017).

Table 1. Range values and statistical distribution of internal constituents quality attributes of fresh goji berries.

Parameters	Min	Max	Mean	Standard Deviation	
Vitamin C (Vitamin C g kg ⁻¹)	0.13	0.65	0.31	0.14	
Ascorbic Acid (AA) (Ascorbic Acid g kg ⁻¹)	0.02	0.48	0.20	0.12	
Dehydroascorbic Acid (DHAA) (DHAA g kg ⁻	0.04	0.18	0.11	0.04	
Antioxidant Activity (Trolox equivalent g kg ⁻¹)	1.63	3.29	2.31	0.41	
Total Phenols (gallic- acid g kg ⁻¹)	2.09	3.37	2.62	0.32	
Anthocyanin (Cyanidin mg cm ⁻²)	0.67	1.35	1.05	0.17	
Soluble Solid Content (SSC) (%)	6.50	25.90	21.62	3.48	
(TA) (%)	0.11	0.92	0.56	0.15	

In this study, the potentiality for both wavelengths ranges in VIS-NIR and NIR for the prediction model of vitamin C, ascorbic acid (AA), dehydroascorbic acid (DHAA), total antioxidant activity, total phenols, anthocyanin, soluble solid content (SSC), and total acidity (TA) are compared. In general VIS-NIR and NIR spectra regions, both are giving reliable predictions of vitamin C, AA, total phenols, SSC, and TA while the prediction for DHAA, is not satisfying (Table 2 and Table 3). However, comparing both regions VIS-NIR and NIR for the prediction of the nutritional value of goji berry, the best performance of prediction for total phenols, SSC and TA are best given in the spectra region of VIS-NIR, whereas the best prediction performance for vitamin C and AA is best given in the spectra region of NIR. It is suggested that the prediction of total phenols is best explained in the visible region since the peaks containing the compounds are in the VIS-NIR region at the range of (400 nm-500 nm) (Bordbar et al., 2017). Particularly it is reported that ferulic acid which is the dominant phenolic of goji berry (Benchennouf, Grigorakis, Loupassaki, & Kokkalou, 2017) shows a maximum reflectance peak at 450 nm (Danial Fatchurrahman, Kuramoto, et al., 2020). Regarding the SSC, the peaks that containing starch, cellulose, and sucrose are also in the VIS-NIR region of 890 nm – 920 nm [28][29]. As for TA, slightly better results were obtained in the VIS-NIR region, indicating that the spectra that contribute to detecting acids are covering both region VIS-NIR and NIR. A previous study reported that citric acid absorbance is represented in the wide region between 900 nm – 1650 nm (Marques, De Freitas, Pimentel, & Pasquini, 2016), while in this study we concluded that most of the information is contained within 900 nm – 1000 nm. Another explanation for this result is that in addition to spectral information directly related to acid content, in the VIS-NIR range other wavelengths are indirectly contributing to detect the acidity, being related to the maturity of the fruit, resulting in better performance for this region, compared to NIR. Also, for vitamin C and AA, both ranges were giving satisfying results, but in this case, NIR results were slightly better performance compared to VIS-NIR region at (400 nm - 1000 nm). This can confirm what already found for rocket leaves, showing that major peaks correlating with vitamin C are in the NIR region (1000, 1210, 1360, 1460, 1580, 1650 nm) while only one peak (850 nm) is found in the VIS-NIR region [32][33].

The PLSR models yielded reliable and satisfying results for the Vitamin C and AA, phenols, SSC, and TA. Different pre-treatments techniques were applied including smoothing, mean centering, 1st and 2nd derivatization, and their combinations (Table 2 and 3). Besides, to improve the performance after the development of these models, the most significant variables were selected based on modified i-PLS for each parameter and tested to an external data set. Thus, the best prediction results are explained in terms of regression R², the root mean square error for calibration (RMSEC), leave one out cross-validation (RMSECV), and root mean square error for prediction (RMSEP) in NIR and Vis-NIR range, respectively (Table 4). Particularly when R² and errors were giving different indications, models with lowest prediction error were selected.

Table 2. Calibration statistics for the PLSR models of the internal constituents of fresh goji berries (SM = Smoothing, Dev = derivative, MC = mean centering) in the VIS-NIR region (400 nm - 1000 nm).

		NI. NI.					RMSE	
Parameter	Pre- treatment	No. Var	No. Sample	LVs	R^2_{Cal}	RMSEC	R^2_{CV}	CV
Vitamin C	SM+MC	121	92	10	0.79	0.05	0.69	0.07
	$SM+1^{st}$	121	92	5	0.60	0.06	0.56	0.07
	Dev+MC	141	92	3	0.00	0.00	0.50	0.07
AA	SM+2 nd	121	92	5	0.84	0.05	0.76	0.07
	Dev+MC	121	92	13		0.03		0.05
	SM+MC SM+1 st			13	0.89		0.64	
	Dev+MC	121	92	7	0.75	0.04	0.64	0.05
	SM+2 nd			_			0.60	
	Dev+MC	121	92	5	0.75	0.04	0.69	0.05
DHAA	SM+MC	121	92	3	0.31	0.03	0.31	0.03
	SM+1st	121	92	1	0.36	0.03	0.40	0.03
	Dev+MC	121)2	1	0.50	0.03	0.40	0.03
	SM+2 nd	121	92	1	0.39	0.03	0.42	0.03
Total	Dev+MC							
Antioxidant	SM+MC	121	97	4	0.21	0.32	0.22	0.35
Antioxidant	SM+1st							
	Dev+MC	121	97	3	0.31	0.30	0.31	0.34
	SM+Log+1s	121	07	3	0.39	0.25	0.37	0.29
	t Dev+MC	121	97			0.25		
Phenols	SM+MC	121	97	2	0.37	0.26	0.40	0.27
	SM+1st	121	97	5	0.36	0.19	0.33	0.23
	Dev+MC							
	SM+Log+2 nd Dev+MC	121	97	2	0.41	0.23	0.44	0.24
Anthocyani								
n	Dev+MC	121	97	4	0.14	0.15	0.15	0.16
	$SM+2^{nd}$	121	07	2	0.10	0.16	0.10	0.17
	Dev+MC	121	97	2	0.18	0.16	0.18	0.17
	SM+Log+2	121	97	1	0.15	0.16	0.16	0.16
	nd Dev+MC	121	<i>)</i>	•	0.15	0.10	0.10	0.10
SSC	SM+1st	121	97	6	0.82	0.92	0.73	1.15
	Dev+MC SM+2 nd							
	Dev+MC	121	97	4	0.79	0.98	0.69	1.25
	SM+Log+1s							
	t Dev+MC	121	97	6	0.85	0.96	0.75	1.2
Т 4	SM+2 nd	121	07	4	0.42	0.00	0.42	0.00
TA	Dev+MC	121	97	4	0.43	0.06	0.43	0.09
	$SM+Log+1^s$	121	97	5	0.52	0.06	0.48	0.07
	t Dev+MC	141	71	J	0.52	0.00	0.70	0.07
	SM+Log+2	121	97	4	0.55	0.06	0.51	0.07
	nd Dev+MC		•					

Table 3. Calibration statistics for the PLSR models of the internal constituents of fresh goji berries (SM = Smoothing, Dev = derivative, MC = mean centering) in the NIR region (900 nm - 1700 nm).

Parameter	Pre-treatment	No. Var	No. Sample	LVs	R^2_{Cal}	RMSE C	R ² _{CV}	RMSECV
Vitamin C	SM+1 st Dev+MC	161	95	11	0.70	0.05	0.67	0.06
	SM+Log+1 st Dev+MC	161	95	17	0.65	0.03	0.64	0.06
	SM+Log+2 nd Dev+MC	161	95	10	0.78	0.04	0.70	0.06
AA	SM+1 st Dev+MC	161	95	13	0.94	0.04	0.81	0.06
	SM+Log+1 st Dev+MC	161	95	12	0.65	0.03	0.54	0.05
	SM+Log+2 nd Dev+MC	161	95	10	0.65	0.04	0.57	0.06
DHAA	SM+MC	161	95	4	0.22	0.03	0.26	0.03
	SM+2 nd Dev+MC	161	95	3	0.20	0.03	0.25	0.03
	SM+Log+1 st Dev+MC	161	95	3	0.21	0.03	0.26	0.03
Total Antioxidant	SM+MC	161	97	11	0.21	0.27	0.22	0.34
	SM+Log+1 st Dev+MC	161	97	3	0.22	0.32	0.27	0.35
	SM+Log+2 nd Dev+MC	161	97	2	0.27	0.33	0.34	0.34
Phenols	SM+1 st Dev+MC	161	97	8	0.39	0.18	0.43	0.22
	SM+Log+1 st Dev+MC	161	97	3	0.41	0.22	0.50	0.24
	SM+Log+2 nd Dev+MC	161	97	4	0.34	0.21	0.42	0.22
Anthocyanin	SM+MC	161	97	1	0.04	0.16	0.05	0.17
	SM+Log+1 st Dev+MC	161	97	1	0.05	0.16	0.06	0.17
	SM+Log+2 nd Dev+MC	161	97	1	0.06	0.16	0.07	0.17
SSC	SM+MC	161	97	10	0.14	1.32	0.16	1.57
	SM+1 st Dev+MC	161	97	7	0.19	1.38	0.22	1.59
	SM+Log+2 nd Dev+MC	161	97	8	0.15	1.38	0.16	1.76
TA	SM+1 st Dev+MC	161	97	3	0.42	0.07	0.54	0.08
	SM+2 nd Dev+MC	161	97	4	0.40	0.07	0.52	0.08
	SM+Log+1 st Dev+MC	161	97	7	0.34	0.07	0.40	0.08

Table 4. Prediction statistics for the best PLSR models of the internal constituents of fresh goji berries in the VIS-NIR region (400 nm-1000 nm) and NIR region (900 nm-1700 nm).

Parameter	Effective Wavelength Range (nm)	No. Sample	No. variables	LVs	R ² Cal	RMSEC	R ² CV	RMSECV	R ² _{pred}	RMSEP
	1475-1495									
Vitamin C	1525-1545	85	18	12	0.86	0.03	0.84	0.04	0.96	0.04
	1600-1650									
	925-945									
	975-1120									
	1175-1270									
AA	1300-1320	85	72	11	0.95	0.02	0.91	0.04	0.97	0.04
AA	1425-1445	63	12	11	0.93	0.02	0.51	0.04	0.77	0.04
	1475-1495									
	1525-1545									
	1600-1620									
Total	425-520	87	73	2	0.77	0.16	0.61	0.17	0.62	0.16
Phenols	725-995	07	13	2	0.77	0.10	0.01	0.17	0.02	0.10
	400-495									
	525-545									
SSC	625-670	89	50	4	0.97	0.75	0.95	0.91	0.94	0.70
	850-895									
	925-970									
	400-445									
Т.	600-620	07	21	3	0.00	0.04	0.07	0.04	0.94	0.04
TA	775-795	87	31	3	0.88	0.04	0.87	0.04	0.84	0.04
	825-895									

As can be seen in Tables 2, 3, and 4 the best model performance was chosen based on the selection of both the lowest RMSEP (C. Zhang et al., 2020) and the highest R². In the case of the Ascorbic Acid (AA), encouraging results over 95 samples were obtained with 12 optimal LV in the NIR range, after pre-treating the data using a combination of smoothing, logarithm, 1st derivative, and mean centering. This pre-processing allowed to obtain the lowest error in cross validation (0.54 g kg⁻¹ with an R² of 0.65), even if a model with a much higher R² could be selected (0.94 but with an error of 0.55 g kg⁻¹). Selecting the most effective 84 wavelengths and removing outlier samples the improved model resulted in 11 LV and enhanced performance in calibration R²Cal 0.97 and, with R²pred 0.91 and RMSEP of 0.04 g kg⁻¹, as shown in figure 3 A. The most effective wavelengths for the prediction of AA were given in (table 4). Compared to a study on the prediction of AA in bell pepper which is the

same family as goji berry, we obtained a better performance where the prediction of AA yielded performance only with R²_{pred} 0.70 with an error of 0.18 g kg⁻¹ (Ignat, Schmilovitch, Fefoldi, Steiner, & Alkalai-Tuvia, 2012). Furthermore, in the case of DHAA, we didn't get a satisfactory result (Table 3), and as a consequence, a little worse performance of the prediction model for vitamin C (sum of AA and DHAA) was reached.

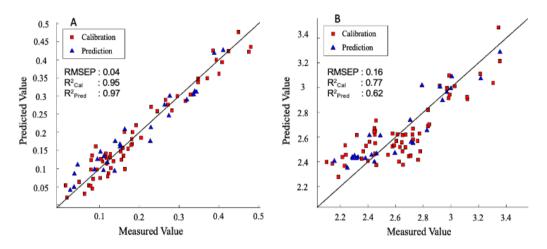


Figure 3. (A) AA model (NIR range) and (B) Total phenols model (VIS-NIR range): PLS regression plot for predicted vs measured values.

In particular, for vitamin C the best calibration model performance was obtained in the NIR range, with a data set comprised of 95 samples, and applying a combination of smoothing, logarithm, 1st derivative followed by mean centering as pre-treatment (Table 3). After selecting the most effective wavelength and removing outlier samples, optimal LVs were reduced to 12 enhancing the performance in calibration and prediction, yielding to R²_{Cal} 0.96 and R²_{pred} 0.91 with RMSEP of 0.04 g kg⁻¹ (Table 4). Furthermore, the most effective wavelengths used for the prediction of vitamin C belonged to the following intervals, 1475-1495 nm, 1525-1545 nm, and 1600-1650 nm. As to the best of our knowledge, there isn't any available report yet for the prediction of vitamin C in goji berry, but comparing to the prediction of vitamin C with other fruit from previous papers, this model gained better accuracy.

As in intact tomato, to be in the same family of Solanaceae, [40] reported the prediction result of R²_{pred} 0.82 and RMSEP of 0.17 g kg⁻¹ by using the whole wavelength range of 930 nm to 1650 nm, while for chili pepper obtained R²cal, R²pred, and RMSEP of 0.95, 0.8 and 0.01 g kg-1 (X. Wang, Xue, He, & Liu, 2011). Also for other fruit, lower performance are reported; for apple obtained an R²_{pred} 0.81 and RMSEP of 0.05 g kg⁻¹ (Pissard et al., 2013), in orange obtained R²_{cal 0.82} and R²_{pred} 0.72 with RMSEP 0.9 g kg⁻¹ (Borba et al., 2020), have been reported, respectively. Regarding total phenols in goji berry, the best model acquired was in the VIS-NIR region, the data set comprised of 95 samples, and the best calibration model was obtained by applying a combination of smoothing, logarithm, 2nd derivative, and mean centering as pre-treatment. Furthermore, selecting the most effective wavelength and removing outlier samples resulted in reducing the optimal LV to 2 and enhancing the performance in calibration and prediction, yielding R^2_{Cal} 0.77 and R²_{pred} 0.62 with RMSEP of 0.16 g kg⁻¹ respectively. The PLS regression plot is given in Figure 3 B. Furthermore, the most effective wavelength range was shown to be in the wavelength intervals of 425-520 nm and 725-995 nm. This result explained that the major information for the most abundant phenolic compounds in goji berry is ferulic acid which has the absorbance in the visible region [36][37]. However, a different result was reported in dried black goji berry (Lycium ruthenicum Murr.) where the prediction was best yielded in the NIR region with the R²Cal, R²pred, and RMSEP of 0.83, 0.84, 3.07 g kg⁻¹ respectively (C. Zhang et al., 2020). As for black goji berry, where the most abundant phenolic compounds are known to be coumaric and caffeic acids [44][45]. The most important peaks in the NIR region fall between 1100 nm and 1170 nm and between 1410 nm to 1480 nm (Han et al., 2017). Besides, it should be noted that Zhang et al (2020) used dried goji berry used by [10], leading to a high concentration of phenolics. As we can observe, despite the higher R² reported by these authors, the RMSEP was higher than in the present study. The error of prediction, is in fact, more important that R² and should be compared to the standard error of laboratory (SEL). As for phenolics, RMSEP was in line with the value calculated for the laboratory destructive measure of total phenols which, in our case is 0.1 g kg⁻¹. It is reported that the value of RMSEP should be considered excellent if it is not higher than 1.5 times the SEL and good if around 2-3 times of laboratory error (Shenk & Westerhaus, 1991) thus, confirming the robustness of the prediction model.

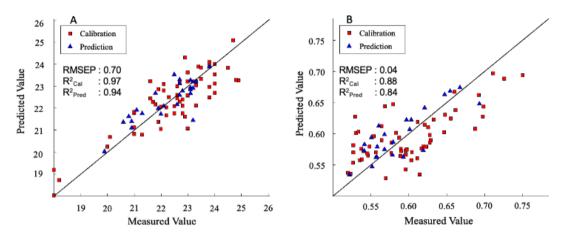


Figure 4. (A) SSC (VIS-NIR range) and (B) TA (VIS-NIR range): PLS regression plot for predicted vs measured values.

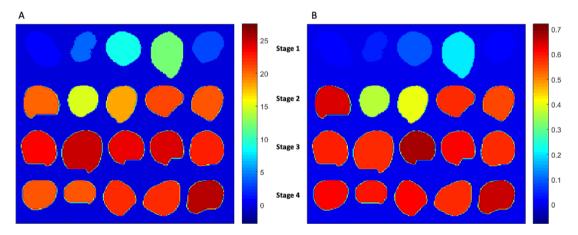


Figure 5. (A) Prediction map of SSC (VIS-NIR range) and (B) TA (VIS-NIR range).

As for SSC, the prediction model comprises 97 samples, elaborated in the VIS-NIR range, combining the smoothing, 2nd derivative followed by mean centering pretreatments. Selecting the most effective wavelengths and removing outlier samples

resulted in enhancing the performance in calibration and prediction yielding R²cal 0.97 and R²_{pred} 0.94 with RMSEP of 0.70. PLS regression plot is shown in Figure 4. A, whereas the most effective wavelengths for the prediction model are shown in (table 4). These results are slightly better than a similar study on the prediction of total sugar content in goji berry, however in this research, the prediction model was done by using FT-NIR spectroscopy and the total sugar was measured by the quantification of glucose ,reaching performance with R²_{Cal} 0.97 and R²_{pred} 0.92 with RMSEP of 0.9 (Li, Yu, & Gao, 2017). Figure 5.A depicts the prediction of the concentration of SSC from fruit over different maturity stages. It can be seen that goji berry fruit has a lower SSC concentration at approximately 4 % in stage 1 and increasing to the level of concentration of 25 % in stage 4 respectively. Regarding TA, the best PLSR model was developed using the VIS-NIR range, combining smoothing, 2nd derivative, and mean centering. Selecting the most effective wavelengths and removing outlier samples resulted in reducing the optimal LV to 3 and enhancing the performance in calibration and prediction (R²Cal 0.89 and R²pred 0.84 and RMSEP of 0.04 %). PLS regression plot for TA is shown in (Figure 4. B). This is the first attempt of TA prediction in goji berry with spectral data. Comparing these results with the prediction of TA in grape tomato, despite the lowest R²_{Cal} and R²_{pred} we had a smallest prediction error (RMSP 0.04% vs 0.072 %) (Sohrabi, Ahmadi, & Monavar, 2018). Furthermore, figure 5 B expresses the concentration of TA in goji berry across 4 different maturity stages. Finally, it needs to be taken into some consideration that the laboratory error of SSC and TA is 0.392 and 0.02, respectively, which is confirming the robustness of the models. Finally, as for total antioxidant activity, and total anthocyanin results were very poor. As for anthocyanins is possible that signal due to the content of these phytonutrients is very low compared to the water and main constituents, since their content is lower even if compared to phenolics and vitamin C. Finally, antioxidant activity is correlated to the content of different compounds but it's the measurement is the results of the addition of a free radical being reduced in an oxidizing medium (Singleton & Rossi,

1965), so it is completely understandable that no direct correlation with reflectance spectra exist since the response is chemically activated in the reference essay.

4. Conclusions

The potential of hyperspectral imaging in both region VIS-NIR and NIR, together with multivariate data analysis was evaluated for non-destructive determination of internal composition of intact goji berry. In general, the result from the prediction model in the VIS-NIR region can be used to predict total phenols, SSC, TA showing better performance than in the NIR region. Despite, unsuitable prediction of DHAA, anthocyanin, and total antioxidant, the results obtained in the current study are very promising for fast quality evaluation of goji berry fruit, since vitamin C, total phenols, SSC and TA are the most relevant parameters related to fresh goji berry composition and consumer acceptance.

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Classification of Common Defective Goji Berry (*Lycium barbarum* L.) by Using Vis-NIR Hyperspectral Imaging Method

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Abstract

Goji berries are widely recognized for their outstanding health benefit and antioxidant properties. The fruit is very susceptible to mechanical damage and physiological disorders leading to depreciate its value and lose its marketability. Hyperspectral imaging (HIS) system over Vis-NIR ranges was used for the detection of common defects. Reference measurement of defects was acquired by visual appearance analysis. The outcomes of this study indicate the promising potential for visible-NIR to provide non-invasive, rapid, and reliable early detection of common disorders in goji berry fruits.

Keyword: hyperspectral imaging, multivariate analysis, PLS-DA, defect, goji berry

1. Introduction

Goji berry (*Lycium barbarum* L.) is widely recognized for its outstanding health benefit and antioxidant properties, a fruit of the family of Solanaceae (Donno et al., 2015; Kulczyński and Gramza-Michałowska, 2016). Originated from Asia and was introduced in Europe in the 18th century for its famous benefits for health and medical properties (Wojdyło et al., 2018). Freshness is a quality attribute that determines the commercial values and sales of goji (Kulczyński & Gramza-Michałowska, 2016). Goji berries are very fragile fruits with a low firmness, which is susceptible to visual damage as a result of mechanical damage during harvest or postharvest handling as the berries leak juice and turn black if they are bruised, or squashed (Kruczek, Ochmian, Krupa-Małkiewicz, & Lachowicz, 2020). Regarding physiological disorders, goji fruit is highly sensitive to black spots, associated with changes in flesh-rind water conditions during storage caused by the dehydration process in the rind (Munera et al., 2021). Another disorder is the development of molds on the skin which is induced when they are exposed to high temperatures before or after harvest and longer storage times due to high respiration rate (Palumbo

et al., 2020).

Sorting goji to eliminate defective berries is a task traditionally carried out manually and requires a lot of efforts. As all the manual operations sorting is affected by subjective and psychological factors such as fatigue, personal habits and by environmental factors as light, belt speed and generally working conditions (Pu, Feng, & Sun, 2015). Therefore, preventing these drawbacks, it would be advisable to consider automatized and objective optical techniques which have been successfully applied for quality assessment and reliable inspection of fresh agricultural and horticultural commodities in the past few years (Beghi et al., 2017; Cen et al., 2016; Chaudhry et al., 2018; Hubo et al., 2017; Kleynen et al., 2005; Yu et al., 2014).

Defect detection based on colour has been already applied in a vast area using machine vision system as a precise and low-cost grading system (Ireri et al., 2019; Lu and Lu, 2018; Mahendran et al., 2015; Sahu and Potdar, 2017) but it has some limits when applied to early detection, before symptoms became visible.

In this regard, hyperspectral imaging (HSI) has the advantage of carrying information about internal compositional and structural changes occurring as consequence of physiological and mechanical damages; it can also estimate chemical constituents in fruit without prior sample preparation (Pu et al., 2015). Bell peppers could be discriminated by sounds fruit using Vis-NIR hyperspectral images (Babellahi, Paliwal, et al., 2020). Additionally, Munera et al. (2021) evaluated HSI combined with machine learning techniques as a method to distinguish common defects in 'Algerie' loquat fruit, such as purple spot, bruising, russeting, or flesh. ElMasry et al. (2007) triumphantly implemented line-scanned HSI in conjunction with PLS-DA to early bruise detection on "McIntosh" apples, even though the colour of a bruised region is similar to the color of the apple surface. (Gómez-Sanchis et al., 2008) studied the feasibility of detecting rottenness in mandarins caused by Penicillium digitatum in the early stages of infection once the rotten area is hardly visible to the human eye by using HSI. The rate of success is above 91%.

Lü and Tang. (2012) examined the Vis-NIR HSI technique to detect hidden bruises on kiwi fruit by using an SVM classifier with a total detection error rate of 14.5%. Vélez Rivera et al. (2014) described HSI to detect invisible damage in the pericarp of 'Manila' mango Yuan et al. used Vis-NIR hyperspectral imaging technology combined with PLS-DA to early detection of internal bruises in jujube fruit. They reported that PLS-DA could discriminate different stages of bruising with high accuracy (Yuan et al., 2021).

To extract and summarise spectral information from hyperspectral images, partial least squares-discriminant analysis (PLS-DA) is widely used to reduce the high dimensionality of the spectral data and to overcome the problem of multicollinearity. Amodio et al. (2017) developed a PLS-DA model to discriminate among strawberry fruit from different production systems. Babellahi et al., (2020a) used PLS-DA as a supervised classification method to discriminate between bell pepper fruit stored at chilling and safe temperatures; and they applied an innovative ANOVAsimultaneous component analysis (ASCA) to study the effect of the experimental factors, as temperature and the storage time, on the changes in the spectral profile of eggplant fruit. Yuan et al. (2021) evaluated PLS-DA to rapidly detect the intact and damaged jujube at five-time points after mechanical damage (2 h, 4 h, 8 h, 12 h, and 24 h). It was found that the model accurately detected bruising in jujube 8 h after bruising, and the accuracy of the calibration set and prediction set was acceptable. Therefore, to the best of our knowledge there has been no report regarding the early detection of several natural defects on fresh goji berry fruit which occurs during storage. Hence, the aim of this study was the early detection of defective goji fruit during storage before fruit are manifested by defects according to their severity: mild damages (i.e. visual damage, softening, bruise), moderate damages (i.e. pitting, and initial mold), and severe damages (i.e. severe mold).

2. Material and methods

2.1. Fruit samples

The total amount of 625 goji berry fruits (Lycium barbarum L.; Cultivar: sweet berry) grown in an open field in the Province of Castellaneta (Italy) were harvested conventionally by picking the fruit with their peduncles. 265 fruits were used for the initial scanning (hyperspectral image scanning) evaluation and having a different degree of defect, in order to have the wider variance of defects the rest of the fruits were split into 3 groups with each group containing 120 fruits subjected to storage at temperatures 0, 5, and 10 °C under a relative humidity of 95 %. The data acquisition days were conducted on day 4th and 8th after harvest with a total of 60 different goji fruits for every scanning day from every temperature storage. Standard quality was measured, including, the incident of disorders, moreover, as for the spectral analysis, hyperspectral images in Vis-NIR ranges were taken.

2.2. Quality assessment

The disorders consisted of fungal infestation, black spot, pitting, softening, shrivelling, cracking, peel disorder, rot, visible flesh damage were categorized following a previous study (Jatoi et al., 2018). Defects were denoted by a human expert on three categories according to their severity (Figure 1): mild damages (i.e. visual damage, softening, bruise), moderate damages (i.e. pitting, initial mold), and severe damages (i.e. severe mold). The amount of incidence was done by a manual calculation and expressed in percent.

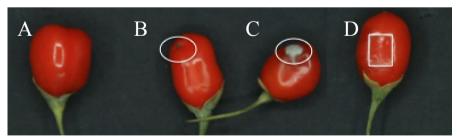


Figure 1. physical appearances of goji berry fruits (A) sound fruit, (B) black spot which is initial mold incident (indicated by a white circle), (C) severe mold incident (indicated by a white circle), (D) visual damage (indicated by a white square)

2.3. Hyperspectral Image Acquisition and Spectra Extraction

Hyperspectral image acquisition was done by using a hyperspectral line-scan scanner (Version 1.4, DV srl, Padova, Italy) consisting of a CCD camera in the visible near-infrared (Vis-NIR) region which has a spatial resolution of 27.9 pixels/mm² with a spectral resolution of 5 nm over a wavelength range of 400–1000 nm. A cooled halogen lamp with a stabilized power source was used as the excitation system. The GigE vision was used as the interface with a 37° field of view (FOV). Image thresholding, masking, and the extraction of the spectra of the pixels were done under MATLAB with a self-developed code. For this purpose, the spectrum of each pixel from the image of the fruit after eliminating abnormal regions altering the spectral fingerprint-like glare of incident light and also defected regions with rapid change in reflectance (Figure. 2) was extracted then the mean spectra of pixels corresponding to the samples was estimated for producing one spectrum for each fruit.

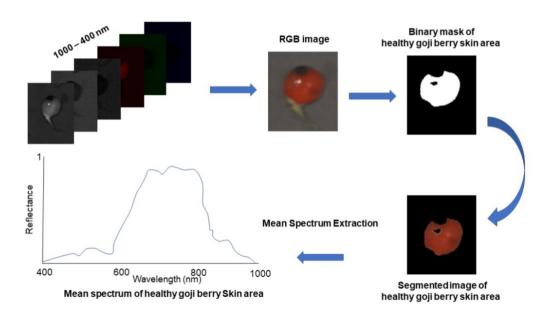


Figure 2. The flowchart of Image elaboration and obtaining mean spectrum

2.4. Chemometric

2.4.1. Principal Component analysis

In this report PCA is used for spectra elaboration. In spectral data analysis PCA is widely used method for feature extraction and spectra elaboration. New orthogonal and uncorrelated variables which is so called as principal components (PCs) are produced from the transformation of original variables, and each PC demonstrates a linear combination of the original wavelengths. The explained number of the total variances ranked the PCs. PCA linearly transforms the original variables into new orthogonal and uncorrelated variables (called principal components, PCs), and each PC is a linear combination of the original wavelengths. The PCs are ranked by the explained amount of the total variances. PC1 explains the most of total variances, followed by PC2, PC3, PC4 and so on. In general, the first few PCs contain the most information of the samples, and the scores of these PCs can be extracted as features (Xu et al., 2018).

2.4.2. Partial least square-discriminant analysis (PLS-DA)

PLS-DA model is an algorithm based on the relation between spectral intensity and sample characteristics (Brereton & Lloyd, 2014). In PLS discrimination, the samples are separated into groups, based on the latent variables estimated from the calibration dataset and the previous knowledge on the existence of sample classes (Olivieri, 2018). PLS-DA derives from partial least squares regression (PLSR). In case of a PLS-DA, a regression model between the X (data acquired from instrument) and Y (dummy binary vector for coded samples) is developed. Classification of the samples is then accomplished based on the values of the predicted Y which, unlike those of the dummy matrix used for model building, are real-valued (Brereton & Lloyd, 2014). In practice the reliability of the model is evaluated in prediction using an external dataset (i.e., samples neither used for modelling nor for model selection), and, simultaneously, to ensure that enough

samples could be used for model development and validation, a repeated double cross-validation (rDCV) strategy was adopted. Double cross-validation (DCV) consists of two cross-validation loops (an inner and an outer loop) nested in one another. The inner cross-validation loop was used for model selection (i.e., for choosing the optimal pre-processing and the number of latent variables) by using 437 fruit, whereas the outer loop contains 188 fruit samples which are in turn treated as external validation sets. To avoid the estimate being biased by a specific division of samples into the different cancelation groups, the whole procedure was iterated for 50 times, hence the term repeated double cross-validation (Filzmoser, Liebmann, & Varmuza, 2009). In particular, different pre-processing methods were tested on the data, i.e., standard normal variate (SNV), derivatives calculated with different number of points and orders of the interpolating polynomial, and their combinations. As stated, for each cancelation group in the outer cross-validation loop, selection of the optimal model (in terms of optimal pre-treatment and number of latent variables) was used to predict the validation samples based on the minimum classification error in the inner CV loop. Second derivative (Savitzky-Golay, 15 points window, 3rd order polynomial) and mean centering served as the best pre-treatment.

3. Results

The physical appearances of goji berry fruits on the harvest day and during storage can be seen in Fig. 1. According to the severity of the deterioration, defects were grouped into four categories: mild damages (e.g. softening), moderate damages (e.g. pitting, initial mold), and severe damages (e.g. severe mold).

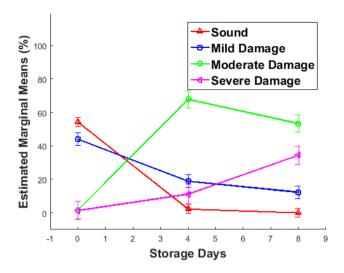


Fig. 2. The incidence of disorders of goji berries during storage day

Fig. 2 depicted the incidence of disorders over storage time. We observed that there was a significant effect of time of storage on the development of severity of the damage, however, difference among temperatures were significant only at 8 days of storage (p<0.05), with fruit stored at 10 °C showing the highest deterioration (p<0.05). Severe damages were noticed to increase over storage in all treatments (from 1.3% to 34.4%) in parallel to a reduction of sound and mild defective fruits, which decreased from 54.3% and 44.0% to 0 and 12.2%, respectively. At the end of the storage 7.8 % of the fruit showed mild damage, 57.8% of the fruit showed moderate damages and 34.4% severe damage.

The classification of 4 classes damage of the fruits were impossible to be done by a simple classification acquired from conventional digital image due to the difficulty on the external appearances of the fruit in the category of mild and medium where fruits shows softening and pitting. For this reason, hyperspectral image is done to be more robust tool to classify among identified defects. For the first spectra elaboration, PCA was conducted following the spectra pre-treatments of mean centering, smoothing, first derivative, second derivative, MSC and followed by

DETREND) and figure 3 depicts the score plot of the PCA of spectra of goji berries labelled using 2 (sound and damaged fruit) and 4 classes (sound, mild, moderate and severe damage). As can be observed, this kind of unsupervised explorative analysis already indicated differences in the spectra, according to the presence and type of defects.

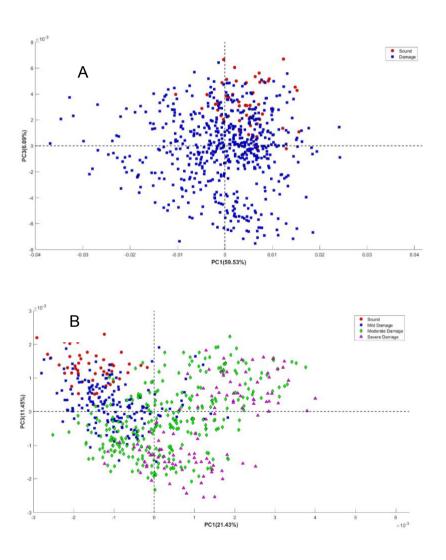


Fig. 3. Score plot of PCA of pre-processed spectra labelling (A) 2 (defective and sound) and (B) 4 classes of damages.

When applying PLS-DA classification model considering two classes only (sound

and defective) gave very satisfying results. As detailed in the Methods section, a classification strategy embedding variable selection through the Covariance Selection algorithm (CovSel) and validated by means of a repeated double cross-validation approach with 10 cancelation groups in the outer loop, 8 cancelation groups in the inner loop and 50 DCV runs was adopted. Prior to model building and validation, data were pretreated by second derivative (Savitzky-Golay, 15 points window, 3rd order polynomial) and mean centering. For each outer loop split (i.e., for each set of external validation samples), the optimal PLS-DA model complexity and the best subset of original variables were selected as those leading to the minimum mean classification error on the inner-loop samples.

When considering the classification ability on the outer loop samples, which can be considered, as already discussed, as external validation samples, the model resulted in a sensitivity of 96.7 ± 1.4 % and 90.7 ± 0.7 % for the "sound" and "defective" categories, respectively. Due to the symmetry of the two-class discriminant problem, the corresponding specificities are 90.7 ± 0.7 % for the "sound" and 96.7 ± 1.4 % for the "defective", corresponding to an overall classification accuracy of 91.2 ± 0.6 % and a mean classification error rate of 6.3 ± 0.8 %.

These results can also be graphically appreciated in Figure 3, where the mean scores of the outer loop samples (over the 50 rDCV runs) across the only canonical variate of the PLS-DA model are displayed together with their 95% confidence interval. It can be easily observed in the plot how almost all the sound samples have scores >0.1, while the defectives have scores lower than that value.

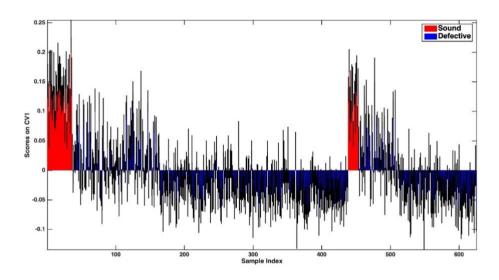


Figure 3. Mean scores plus confidence intervals of the outer loops samples.

These results indicate not only that the model can predict very accurately whether the fruit is sound or presents any defect, but also that such predictions are highly robust and consistent, and do not depend relevantly on the subset of samples used for training. This is one of the advantages of the rDCV approach: it does not only provide point estimates of the classification accuracy on test samples, but also confidence intervals which allow to estimate the stability of the obtained results. This is also true when considering the variable selection embedded in the proposed strategy: indeed, since for each outer loop split a different model is calculated, resulting in a specific set of selected variables, it is also possible to evaluate the consistency of the subset of predictors identified as relevant. In the present study, 14 variables (graphically displayed in Figure 4 A) were selected in more than 80% of the cases, i.e., in at least 400 out of the 500 (50 runs x 10 outer loop splits) calculated models. To further test the efficiency of the selection, a PLS-DA model was built using only those 14 variables and validated through the same rDCV strategy, and a comparable classification accuracy was obtained.

As already commented in the case of the two-class problem, the predictions appear

to be highly consistent, and not to depend relevantly on the subset of samples used for training. Similarly, when considering the variable selection stage embedded in the classification strategy, 14 variables (graphically displayed in Figure 4 B) were selected in more than 80% of the cases, i.e., in at least 400 out of the 500 (50 runs x 10 outer loop splits) calculated models. To further test the efficiency of the selection, a PLS-DA model was built using only those 14 variables and validated through the same rDCV strategy, and a comparable classification accuracy was obtained.

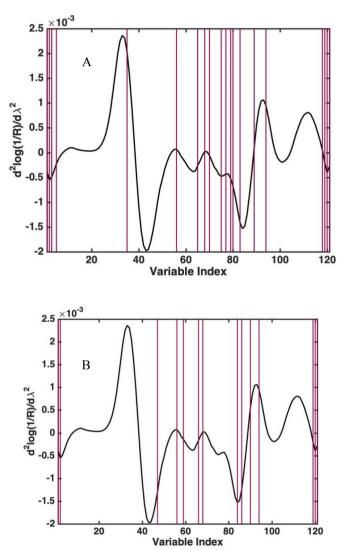


Figure 4. Variable loading plot for classification based on 2 classes (A) and 4 classes (B).

In the first stage of analysis the calibration and prediction results (confusion matrix) for the PLS-DA conducted on a dataset of 625 samples to discriminate between the defected fruit and also the sound fruit (Table 1 and 2). The model gained a high accuracy as 96.7±1.4% the sound and 90.7±0.7 of defected fruit were correctly classified in the external validation set leading to overall accuracy of 91.2±0.6%. In a second stage of the analysis (Table 3 and 4), a classification considering the different levels of defectiveness was also tried. In particular, on the same samples 4 categories were defined: "sound", "mildly defected", "moderately defective" and "severely defective". With this definition of the class labels, model building and validation was carried out as in the case of the two-class problem. In particular, data were pretreated by second derivative (Savitzky-Golay, 15 points window, 3rd order polynomial) and mean centering, a repeated double cross-validation approach with 10 cancelation groups in the outer loop, 8 cancelation groups in the inner loop and 50 DCV runs was adopted to validate the results of CovSel-PLSDA and the selected variables.

Furthermore, for the classification of 4 classes and considering the classification ability on the outer loop samples, the model resulted in a sensitivity of 89.8±2.1 % and a specificity of 92.0±0.6 % for the "sound" category, 62±2 % sensitivity and 88.9±0.7 % specificity for the "mildly defective", 68.2±1.5 % sensitivity and 84.1±1.0 % specificity for the "moderately defective", and 77.6±1.5 % sensitivity and 91.5±0.6 % specificity for the "severely defective". As expected, when looking at the distribution of wrong predictions, they encompass "neighboring" severity grades. The 5.2 wrongly classified sound individuals are classified as mildly defective; analogously, the misclassified individuals from the "mild" category are almost equally mispredicted as "sound" (38.9) or "moderately defective" (30.3). As for the moderately defective pixels, the classification errors are shared between being predicted as "mildly and severly" both at (44) or "sound" (2.6) defective, whereas the severly defective ones are only mispredicted as moderately defective (24).

4. Discussion

By the application of rDCV, the external validation of the PLS-DA model was repeated on the outer loop samples for 500 times. This allowing a point estimate of the predictive ability and corresponding confidence of the model to be obtained, resulting the robust classification accuracy. The results on this study can be considered to be acceptable since the HSI system coupled with PLS-DA is capable of detecting stages of defects without considering of pixels belong to defected regions. Besides, these results are comparable with other recent works carried out using HSI for early damage detection in other fruits. For instance, (Huang et al. (2020). confirmed the fact that an HSI system over the Vis-NIR ranges provided encourages results to recognize early disease blueberry by using PLS-DA. The accurate discrimination results for both healthy and early disease blueberries, with a non-error rate of more than 0.98 accuracies in the calibration set and over 0.95 in the CV and prediction dataset, suggested that the established PLS-DA models were stable and robust (Huang et al., 2020). Yuan et al. (2021) classified early detection of an internal bruise in Lingwu long jujube using HSI coupled with PLS-DA classifier in Vis-NIR ranges and reported maximum the accuracy of the calibration set and prediction set was 85.56% and 92.22%, respectively.

In this study the classification model was best obtained when considering only 2 classes (sound and defective) as high prediction accuracy was obtained with the overall prediction accuracy of 91.2 % and 6.3% error as only 2 individuals are misclassified as defected in the sound class and 53 individuals are misclassified as sound class. Whereas the classification model for predicting 4 classes of fruit based on the defect's occurrence (sound, mild, moderate and severe), the model gives a lower prediction accuracy of 69.7 % with the error of 25.6 %. Observing the error of the model, as discussed before that the distribution of wrong prediction pervades the neighbouring class as for the sound fruit are mispredicted as mild category, and the misclassified of mild category was from moderate and sound, while moderate misclassification was from mild and severe while misclassified of severe was from

moderate. The classification error of the both models (2 and 4 classes defects) was most probably due to the fact that the individual fruit that are wrongly predicted belong to sampling day 4th and 8th where defects are still developing during storage and resulting a misclassification on the prediction. This is clearly shown by the fact that the mispredicted individuals from both (2 and 4 classes) model are fruits from day 4th and 8th of storage. This result is in accordance with our result that the fruit develop defects during storage following the order of visual damage which is pitting and softening found more under 0 °C to black spot as initial mold to severe mold found more on the fruit stored under higher temperature storage (i.e 5 and 7 °C) (Fatchurrahman in press).

Table 1 – PLS-DA Results for the two-class problems. The percentages are calculated on the samples of the outer loop in rDCV (external validation samples)

	LVopt	Classification error (%)	Accuracy	Sensitivity (%)	Specificity (%)
Sound Defected	15±2	6.3±0.8	91.2±0.6	96.7±1.4 90.7±0.7	90.7±0.7 96.7±1.4

Table 2 – PLS-DA Confusion matrix for the two-class problems. The numbers of misclassified individuals are calculated on the samples of the outer loop in rDCV (external validation samples)

		Predicted class			
		Sound	Defected		
True along	Sound	49.3±0.7	1.7±0.7		
True class	Defected	53.3±3.8	520.7±3.8		

Table 3 – PLS-DA Results for the four-class problems. The percentages are calculated on the samples of the outer loop in rDCV (external validation samples)

	LVopt	Classification error (%)	Accuracy	Sensitivity (%)	Specificity (%)
Sound Mild Moderate Severe	19±1	25.6±0.9	69.7±0.9	89.8±2.1 62.0±2.0 68.2±1.5 77.6±1.5	92.8±0.6 88.9±0.7 84.1±1.0 91.5±0.6

Table 4- PLS-DA Confusion matrix for the four-class problems. The numbers of

misclassified individuals are calculated on the samples of the outer loop in rDCV (external validation samples)

		Predicted class					
		Sound	Mild	Moderate	Severe		
	Sound	45.8±1.1	5.2±1.1	0.0 ± 0.1	0.0 ± 0.0		
True	Mild	38.9 ± 2.7	112.8 ± 3.7	30.3 ± 2.6	0.0 ± 0.1		
class	Moderate	2.6 ± 1.5	44.0 ± 3.2	194.3 ± 4.3	44.0 ± 2.8		
	Severe	0.0 ± 0.0	0.0 ± 0.0	24.0 ± 1.6	83.0 ± 1.6		

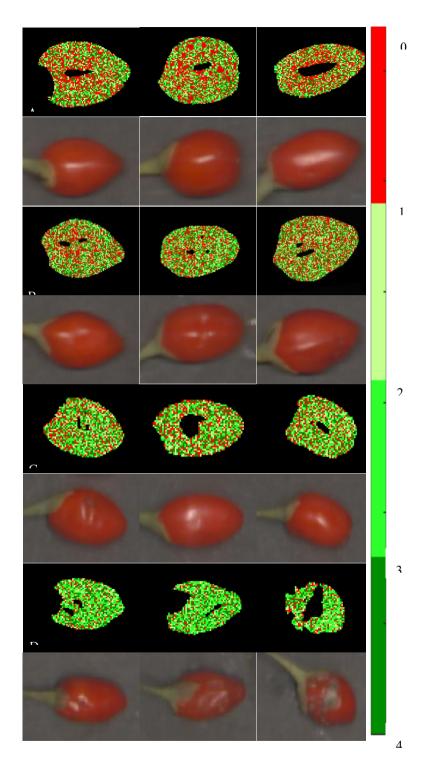


Figure 5. Prediction Image of 4 different class of damages in goji berry fruits: (A) sound, (B) mild damage, (C) moderate damage, and (D) severe damage.

As depicted in figure 5 the result of the prediction mapping of goji berries was classified into 4 classes. Random fruit was chosen from the replicates of each treatment, to visualize the segmentation of disorders over the storage period and temperature levels, where each row represents fruits from each acquisition interval of storage day and each column demonstrates fruits from each acquisition interval of temperature levels. As can be observed, the pixels of each fruit classified into four categories. These results are in agreement with our observation of the severity of disorders in storage duration under different levels of temperature. Prediction of sound fruit consistently improved when the 2-class model was applied.

5. Conclusion

In this work, hyperspectral imaging combined with PLS-DA classifier has been evaluated as a method to early distinguish common defective fruits in goji berry grouped into three categories according to the severity of the deterioration, such as mild damages (e.g. visual damage, softening), moderate damages (e.g. pitting, initial mold), and severe damages (e.g. severe mold). By applying ANOVA-SCA, it was found that the severity of disorders affected the spectral profile of goji fruits and also the most significant wavebands were isolated and used to build a PLS-DA for discrimination. It was concluded that the Vis-NIR range was effective for the classification of the common defects with sound class yielding accuracies as high as 94% and in the case of discriminating between sound and all defective fruits it was as high as 90%.

These results indicate the potential of the proposed methodology based on hyperspectral imaging as a promising tool to assess the quality of goji berry fruits which can enable the real-time discrimination of optically in a sorting line without assessing the whole surface of fruits.

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General Conclusion

Maintaining the fruits quality and prolonging the storage life are post-harvest purpose, and storage condition is the key factor of success. This study described the postharvest handling for goji berry fruit as well as determining the non-destructive and rapid methods for the prediction of quality in term of nutritional constituents as well as physical quality corresponding to common damages. Our studies found that goji fruit could be classified as climacteric fruit, showing a climacteric peak in the early stages of development. Goji fruit harvested at 6 different development stage up to full ripe were characterized in term of metabolic behavior, physical and chemical characteristics. We found that fruit dimension (length, width and weight), and soluble solid content increased from class 1 to 6. The soluble solid content of goji berry from class 4 to 6 was stabilized around the maximum value of 23 %, at harvest or during eight days of storage at room temperature, for the earliest stages. Their high nutritional value was confirmed by vitamin C content at maximum value of 0.52 g/kg. Despite finding climacteric peak already at stage 1-2, only fruit of class 4 were able to reach the maximum level of SSC after being detached from the plant. So, we defined this stage as physiological mature, at harvest possessing dimension of weight, width and length at 0.51 g \pm 0.085, 9.40 mm \pm 0.694, and 11.82 mm respectively. Additionally, aat this stage the fruit developed already the maximum red color at harvest As for Storage temperature, we showed that goji fruit are sensitive to chilling injuries when stored at 0 °C, indicating 5 °C as optimal storage temperature. At this temperature, we observed the lowest level of physiological disorders, while preserving physical, sensorial and nutritional quality attributes, with a storability of about 9 days.

In order to extend shelf-life of goji berry the effect of controlled atmosphere storage (CA) with high CO2 concentrations (form 5 to 20%) was studied. Air enriched with 20% CO₂ best preserved fruit quality in terms of physical, sensorial, and phytochemical attributes, controlling mold development. Fruit maintained fresh

appearance for about 20 days at 5 °C of storage, obtaining a sensible increase of the shelf-life in comparison to air and atmosphere with 5 % and 10 % CO2 which showed higher incidence of damage and storage disorders, particularly for decay. These results are of crucial importance for designing modified atmosphere packaging and easy goji fruit distribution and marketing.

The potential of hyperspectral imaging in both region VIS-NIR and NIR, together with multivariate data analysis was evaluated for non-destructive determination of internal composition of intact goji berry and for sorting defective fruit. The results obtained were very promising for fast quality assessment of content of vitamin C, total phenols, SSC and TA, which are the most relevant parameters related to fresh fruit composition and consumer acceptance. Additionally, PLS-DA classifier has been evaluated as a method to early detect defective fruits grouped into three categories according to the severity of the deterioration, such as mild damages (e.g. visual damage, softening), moderate damages (e.g. pitting, initial mold), and severe damages (e.g. severe mold). It was concluded that the Vis-NIR range was very effective for the classification of sound and defective fruit with a lower accuracy in case of a model with 4 classes.

All these results can allow a sensible improvement in the logistic and processing chain of fresh goji berry, ensuring high quality standards, by optimizing storage conditions from one side and providing tool for online sorting a selection of defective fruit, from the other size. Moreover, developed non-destructive models could allow to sort only sound fruit, while providing at the same time, quantitative information on, total acidity, soluble solid, vitamin C, and total phenol content.