

Modified atmosphere packaging to improve the shelf-life of Goji berries during cold storage

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abbreviation: AA= Antioxidant activity; AIR= Control in air; CIE= Commission Internationale de l'Eclairage; EtOH= Ethanol; fw= fresh weight; MAP= modified atmosphere packaging; MeOH= Methanol; pMAP= passive modified atmosphere packaging; RR= Respiration rate; TP= Total phenols; TSS= total soluble solids; VQ= visual quality.

Abstract: This study was carried out to evaluate the effect of modified atmosphere packaging on the quality parameters and the shelf-life of fresh goji berries. Fruits, placed in trays, were closed in passive modified atmosphere packaging (pMAP) using polypropylene bags or kept in open polyethylene bags (AIR) as control. Samples were analyzed just after harvest and during storage (5, 13 days) at 7°C for visual quality (VQ), color parameters, weight loss, dry weight, total soluble solids (TSS), antioxidant activity (AA) and total phenols (TP), while respiration rate (RR) was evaluated only after 5 days. Changes in gas composition in pMAP samples was measured daily. The use of pMAP allowed to reduce the RR of about 26% compared to fresh sample, to preserve the berries weight loss during storage and their marketability until 13 day at 7°C, while AIR samples were not edible after 5 days due to mold growth on the berries surfaces. No changes of color parameters, dry weight, TSS, AA and TP were observed during storage comparing treatments. In conclusion, the use of pMAP was able to extend the shelf-life of goji berries for 13 days at 7°C, 8 days more than berries stored in AIR.

1. Introduction

The goji (*Lycium barbarum* L.) berries, also known as wolfberries, are considered “superfruits” for their high nutritional value, richness in nutrients, antioxidants and bioactive compounds of which the health promoting properties are known (Sidhu and Zafar, 2012; Jatoi *et al.*, 2017; Niro *et al.*, 2017). The berries are mostly grown for dry fruit, but nowadays the

goji market is significantly expanding, focus also on the fresh fruit. However, due to the tender peel and high-water content, fresh goji berries are easy to damage and rot, so their transport and storage are difficult (Fan *et al.*, 2019). For these reasons the use of postharvest handling to preserve the storability of this perishable fruit are required. Very few studies are been conducted on the storage of fresh goji berries. Jatoi *et al.* (2018) evaluated the postharvest quality of fresh goji berries stored at different temperature, from -2°C to 20°C, concluding that the optimum storage temperature to preserve phytochemical and sensory attributes was 0°C. The application of the lecithin (Jatoi *et al.*, 2017) or edible coating based on lotus leaf extract (Fan *et al.*, 2019) were studied to improve the shelf-life of fresh goji berries. Ban *et al.* (2015) reported that a combination of heat treatment at 40°C for 30 min followed by chitosan coating protect goji berries from decay, extending their postharvest life up to 28 days of storage at 2°C. The use of additives or coatings, even though are natural, is often undesirable from consumers that are more attracted to fresh products without any additional ingredients. From this point of view, the use of modified atmosphere packaging (MAP) during storage, consisting in a reduction of O₂ and/or an increase in CO₂ levels, can be a valid tool, in addition to the proper temperature, in order to improve the shelf-life of goji berries. Modified atmosphere act reducing respiration rate and weight loss, delaying ripening and softening, thus minimizing the incidence of some physiological disorders and decay (Kader, 2002 a). Kafkaletou *et al.* (2017) tested the effectiveness of a short-term treatments with different atmospheres enriched in CO₂ to prevent fungal decay in fresh goji berries, concluding that atmospheres with high CO₂, from 15 to 20%, were able to reduce fungal decay incidence in goji berries stored for 14 days at 1°C. To the best of our knowledge, no further studies on the application of modified atmosphere on goji berries are available, so the present study is aimed to evaluate the application of MAP technology to extend the shelf-life of fresh goji berries.

2. Materials and Methods

Reagents

Extraction solvents (MeOH, EtOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and all standards used in the experiments were obtained from

Sigma-Aldrich (St. Louis, Mo., USA). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany).

Plant material and experimental set-up

Fresh goji berries (*Lycium barbarum* L.), about 2 kg, were provided from Favella group located in the South of Italy (Corigliano Calabro, Italy), and transported in cold condition to the Postharvest Laboratory of CNR-ISPA to be processed. After elimination of damaged fruits, 3 replicates of about 80 grams berries were used for the initial determination, while the remaining samples were placed in polyethylene terephthalate trays (model C250/50 Carton Pack®, Italy), 80 grams per trays, and sealed in passive modified atmosphere packaging (pMAP) using polypropylene bags (dimension 200 x 150 mm, 30 µm thickness), or in unsealed polyethylene bags (AIR) as control. For each packaging condition (pMAP or AIR), 6 bags (3 replicates × 2 storage times) were stored at 7°C (±1) and analysed initially and after 5 and 13 days for visual quality, color parameters, weight loss, dry weight, total soluble solids, antioxidant activity and total phenols, while respiration rate was evaluated initially and after 5 days, because of mould development in AIR samples after 13 days. In addition, changes in gas composition in pMAP samples was monitored daily using a gas analyser (CheckPoint, PBI Dansensor, Ringsted, Denmark).

Respiration rate

The respiration rate of goji berries was measured at 7°C using a closed system as reported by Kader (2002 b). About 80 grams of berries for each replicate were put into 6 L sealed plastic jars (one jar for replicate) where CO₂ was allowed to accumulate until the value of 0.1%. The time needed to reach this value was calculated, making CO₂ measurement at regular time intervals. For the CO₂ analysis, 1 mL gas sample was taken from the head space of the plastic jars through a rubber septum and injected into the gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA) equipped with dual columns and thermal conductivity detector. CO₂ was analyzed with a retention time of 16 s and total run time of 120 s on a 10 m porous polymer (PPU) column at a constant temperature of 70°C. Respiration rate was expressed as mL CO₂ kg⁻¹ h⁻¹. After the respiration rate evaluation, berries were used for the following analysis.

Visual quality and color analysis

Visual quality was evaluated by a group of ten

trained people, on a subjective 5 to 1 scale, with 5= excellent, no defects; 4= very good, minor defects; 3= fair, moderate defects; 2= poor, major defects; 1= inedible. A score of 3 was considered to be the limit of marketability, while a score of 2 represented the limit of edibility.

Color parameters (L^* , a^* and b^*) were measured, for each replicate, on 3 random points on peel surface of 5 goji berries using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in the reflectance mode and in the CIE $L^* a^* b^*$ color scale. Colorimeter was calibrated with a standard reference having values of L^* , a^* and b^* corresponding to 97.55, 1.32 and 1.41, respectively. Hue angle ($h^\circ = \arctan b^*/a^*$) and saturation ($Chroma = \sqrt{a^{*2} + b^{*2}}$) were then calculated from primary L^* , a^* and b^* readings.

Weight loss, dry weight and total soluble solid content

Goji weight loss was calculated at each storage time as percentage of variation from the initial fresh weight. To measure dry weight, goji berries were maintained in a forced-draft oven at 65°C until constant weight was reached. Total soluble solid content, expressed in °Brix, was measured using a digital refractometer (model DBR35, XS Instruments, Carpi, Italy) on a liquid extract obtained by whisking in a blender (1 min; 14,000 rev. min⁻¹) 10 goji berries from each replicate and then filtering the juice.

Antioxidant activity and total phenols

To determine both antioxidant activity and total phenol contents, the extraction procedure reported by Cefola *et al.* (2012) was followed. In detail, 5 grams samples were homogenized (Ultraturrax T-25, IKA Staufen Germany) in a MeOH:water (80:20) solution for 1 min, and then centrifuged at 5°C at 6440 × *g* for 5 min. The supernatant was therefore used for the assays. The antioxidant activity assay was performed following the procedure described by Brand-Williams *et al.* (1995) with minor modifications. Briefly, the supernatant, proper diluted, was pipetted into 0.95 mL of DPPH solution to start the reaction. The absorbance was read after about 30 min at 515 nm. Trolox was used as a standard and the antioxidant activity was expressed in milligrams of Trolox per 100 g of fresh weight (fw) (mg Trolox 100 g⁻¹ fw). The total phenol content was determined according to the method of Singleton and Rossi (1965). Each extract (100 µL), proper diluted, 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 after 2 h at 765 nm. Total phenol content was calculated on the basis of the cali-

bration curve of gallic acid and expressed as milligrams of gallic acid per 100 g of fresh weight (mg gallic acid 100 g⁻¹ fw).

Statistical analysis

In order to evaluate the effect of packaging condition (pMAP or AIR) on quality parameters of goji berries, a one way ANOVA was performed at each storage time (5 and 13 days), and mean values were separated applying Least Significant Difference (LSD) Multiple Range Test with significant difference when $P \leq 0.05$.

3. Results

At harvest, the respiration rate of goji berries was 23.6 (±3.5) mL CO₂ kg⁻¹ h⁻¹ at 7°C. After 5 days of storage, respiration rate in AIR samples slightly increased, while the use of pMAP allowed to reduce the rate of respiration of about 26% compared to fresh sample (Table 1).

In figure 1 changes in gas composition inside bags in pMAP samples were reported. Starting from air composition (21% O₂ and 0.03% CO₂), oxygen was gradually consumed by the product, due to the respiration process, with a consequent accumulation of CO₂. At the first sampling time, after 5 days at 7°C, O₂

Table 1 - Respiration rate of goji berries at harvest and after 5 days at 7°C (±1) in pMAP or AIR

Respiration rate (mL CO ₂ kg ⁻¹ h ⁻¹)	
<i>At harvest</i>	
Fresh	23.6 ± 3.5
<i>After 5 days at 7°C</i>	
pMAP	17.3 ± 0.2 b
AIR	28.4 ± 1.9 a

Values are means of three replicates ± standard deviation. Different letters indicate statistical differences for $P \leq 0.05$, according to LSD test.

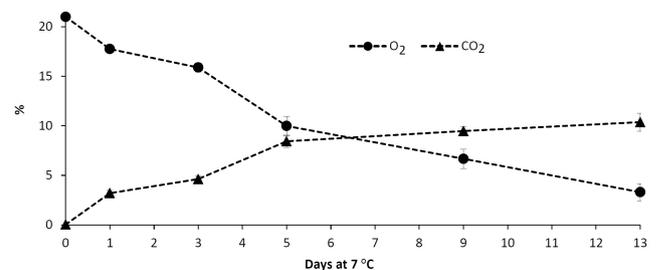


Fig. 1 - Changes in gas composition of goji berries stored in pMAP for 13 days at 7°C (±1). Values are means of three replicates for each storage time ± standard deviation.

and CO₂ values were 10.0% (± 1.0) and 8.4% (± 0.6), respectively. Then, the consumption of oxygen was slowdown, reaching the concentration of 3.3% (± 0.9) after 13 days at 7°C, while CO₂ was 10.4% (± 0.9) (Fig. 1).

The visual quality of the product at harvest was not optimal, in fact panelists gave an initial score of 4 (good) (Fig. 2). At each storage time, significant differences between treatments were observed. In particular, after 5 days at 7°C goji berries stored in AIR were not edible, mainly due to mold growth on the berries surfaces, while pMAP samples were scored as more than acceptable, keeping their marketability until the end of the storage (Fig. 2).

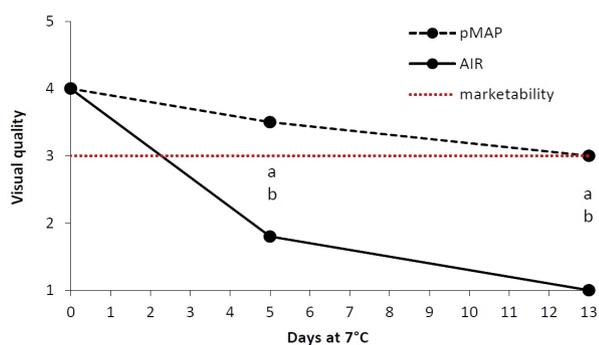


Fig. 2 - Changes in visual quality of goji berries stored in pMAP or AIR for 13 days at 7°C (±1). Values are means of three replicates for each packaging condition at each storage time. Within the same storage time, different letters indicate statistical differences, P≤0.05. Visual quality score: 5=excellent, no defects; 4=very good, minor defects; 3=fair, moderate defects; 2=poor, major defects; 1=inedible.

The weight loss of goji berries stored in pMAP was almost constant for all the storage period while fruits stored in AIR lost about 17.5% of the initial weight after 13 days at 7°C (Fig. 3).

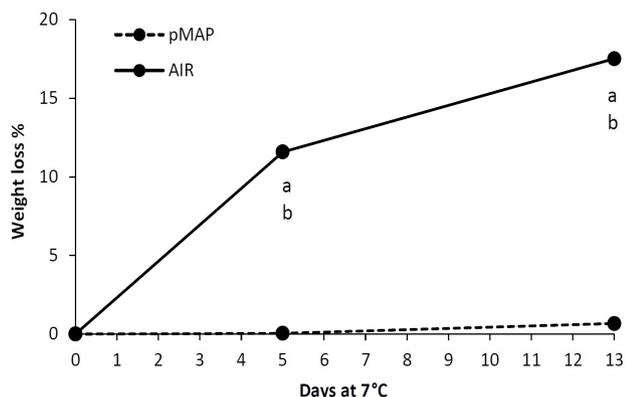


Fig. 3 - Weight loss of goji berries stored in pMAP or AIR for 13 days at 7°C (±1). Values are means of three replicates for each packaging condition at each storage time. Within the same storage time, different letters indicate statistical differences, P≤0.05.

Regard the other physical (color, dry weight) and chemical (total soluble solid content, antioxidant activity, total phenols) parameters, the values measured at harvest are reported in Table 2; however, no significant changes were observed during storage and comparing treatments (at the end of the storage dry weight, TSS, AA and TP had mean values of 23.9%±0.6, 21.7±0.4°Brix, 82.1±2.4 mg Trolox 100 g⁻¹ FW and 216.1±1.3 mg gallic acid 100 g⁻¹ FW, respectively), except for all the color parameters that slightly decreased during storage (at the end of the storage, L*, a*, b*, h° and Chroma mean values were 46.8±0.7, 38.4±0.1, 37.3±0.1, 14.0±0.3, 4.8±1.3, respectively).

Table 2 - Color parameters, dry weight, total soluble solid content, antioxidant activity and total phenols evaluated in goji berries at harvest

Evaluated parameters	Data
Color parameters	
L*	49.1±0.6
a*	46.9±2.4
b*	48.2±2.6
h°	45.1±0.3
Chroma	67.2±3.5
Dry weight (%)	22.8±0.3
Total soluble solid content (° Brix)	21.4±0.5
Antioxidant activity (mg Trolox 100 g ⁻¹ fw)	85.8±1.5
Total phenols (mg gallic acid 100 g ⁻¹ fw)	211.1±2.4

Values are means of three replicates ± standard deviation.

4. Discussion and Conclusions

Respiration rate represents one of the most important parameters that should be taken into account for the study of the postharvest performance of fresh produce since it is inversely correlated with shelf-life and thus, it can be used as an indicator of perishability. Considering the initial respiration rate of goji berries (23.6±3.5 mL CO₂ kg⁻¹ h⁻¹ at 7°C), these fruits have a high respiratory metabolism, according to the classification reported by Kader (2002 c). In the proposed pMAP, the O₂ reduction and CO₂ accumulation, due to the high berries respiration rate and the permeability properties of the packaging, slowed down the rate of respiration of goji berries. This positive effect of MAP was previously reported on different fruit by Sandhya (2010). Similar results on goji berries treated with different atmospheres of low oxygen and high CO₂ were

reported by Kafkaletou *et al.* (2017). The positive effect of the application of pMAP was reported also on the weight loss that was significant lower in pMAP compared to AIR samples. This because the use of packaging represents a barrier to vapor diffusion which allows to maintain an adequate relative humidity within the package, so tissue dehydration is limited (Zagory and Kader, 1988).

The presence of fungal decay was the main factor that influenced the loss of marketability of fresh goji berries during storage. The CO₂ accumulation (until 10%) inside the packages was able to inhibit the mold growth, as previously observed (Kafkaletou *et al.*, 2017). In particular these Authors reported that an atmosphere with 15-20% of CO₂ applied as a short-term treatment for 2 days at 1°C was able to reduce fungal decay incidence in goji berries stored for 14 days at 1°C.

The red color of goji berries was not influenced by packaging condition (pMAP or AIR), whereas a slight decrease in all the color parameters measured were observed during storage. Similar results were reported on goji berries by Kafkaletou *et al.* (2017) and Jatou *et al.* (2017). Regarding to dry weight, our data (22.8%±0.3) are in accordance with data reported by Niro *et al.* (2017) that found a moisture of 77.4% on fresh goji berries, means 22.6% dry weight. Also, the presented data of total soluble solids at harvest (21.4±0.5°Brix) are similar to that reported by Kafkaletou *et al.* (2017) (from 21 to 25°Brix) and Fan *et al.* (2019) (about 22°Brix). Goji berries is considered a “superfruit” for their antioxidant activity due to the high content of bioactive compounds and vitamin C (Sidhu and Zafar, 2012; Jatou *et al.*, 2017; Niro *et al.*, 2017). In the present research paper, the value of total phenols (211.1±2.4 mg gallic acid 100 g⁻¹ fw) at harvest, is quite similar to that reported in literature on fresh goji berries by Donno *et al.* (2016) (from 199.5 to 240.3 mg gallic acid 100 g⁻¹ fw, depending on region of cultivation) and Jatou *et al.* (2017) (about 223 mg gallic acid 100 g⁻¹ fw). As for antioxidant activity, our data at harvest were similar (85.8±1.5 mg Trolox 100 g⁻¹ fw) to data reported by Jatou *et al.* (2017) on the same fruit. Both total phenols and antioxidant activity remain unchanged during storage in pMAP and in AIR samples, as previously reported by Jatou *et al.* (2017) for total phenols.

Fresh goji berries are highly perishable fruits, with a high respiration rate, if stored in air. The loss of marketability is very fast, and it is mainly due to the development of molds on the peel surface. In addition, the high transpiration of the peel causes a rapid

weight loss, with the consequence depreciation of the product. On the other hand, the application of a modified atmosphere packaging resulted a valid tool to delay the loss of quality of goji berries, prolonging their shelf-life. Results of the present study demonstrated that the use of pMAP allowed to reduce the respiration rate, preserved the berries weight loss and the health properties, and control the mould development. As consequence berries goji stored in pMAP showed a shelf-life of 13 days at 7°C, 8 days more than berries stored in AIR.

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