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Carotenoid content of Goji berries: CIELAB, HPLC-DAD analyses and quantitative correlation

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ABSTRACT

Fruits of *Lycium barbarum* L. have been used in Chinese traditional medicine for centuries. In the last decade, there has been much interest in the potential health benefits of many biologically constituents of these fruits. The high level of carotenoids offers protection against development of cardiovascular diseases, diabetes and related comorbidities.

In the present work two different selections of *Lycium barbarum* L., cultivated in Italy and coming from three discrete harvest stages, were subjected to two different grinding procedure and to a simplified extraction method of carotenoid component. CIELAB colorimetric analysis of the freshly prepared purees and HPLC-DAD analysis of carotenoid extracts were performed and compared. Different harvesting dates and grinding procedures deeply influence the carotenoids content and statistical analysis showed high correlation between carotenoid content and colorimetric data.

The final model provides a reliable tool to directly assess carotenoid content by performing cheap and routinely colorimetric analyses for food industry.

1. Introduction

Today foods are not intended to only satisfy hunger and provide necessary nutrients for humans, but also to prevent nutrition-related diseases. Degenerative illnesses, such as cardiovascular diseases, diabetes and relative comorbidities represent the leading causes of death in all industrialised countries. For this reason, consumers are oriented toward the consumption of food with highly recognised health properties, seeking for a better lifestyle in the prevention of these pathologies (Alissa & Ferns, 2017).

In this context, *Lycium barbarum* L. fruit (Goji berry, GB) actually represents the focus of many scientific studies aiming to evaluate its content in bioactive and promoting health components. Goji berries are a traditional Asian food, being China the world's largest producer (Amagase & Farnsworth, 2011). In nature, more than 70 different species of *Lycium* exist, among which *Lycium chinense* (Kafkaletou et al., 2018) is more common but less valuable respect to *Lycium barbarum*, the most appreciated for its phytochemical composition (Dong, Wang, Zhu, & Wang, 2012; Potterat, 2010) and its antioxidant and radical scavenging properties.

Traditionally used in the Chinese medicine for liver protection and antioxidant purpose, GBs are also recommended as food supplements for their promising antiaging and cancer preventive role, cardiovascular protection and restorative activities on immune system functionality (Cheng et al., 2015). The health benefit potential of this fruit requested investigations of its chemical composition thus leading to the identification of polysaccharides, monosaccharides, organic acids, proteins, flavonoids and derivatives, carotenoids, vitamins and mineral salts. (Jin, Huang, Zhao, & Shang, 2013; Mocan et al., 2018). The main component of berries (about 51%) is represented by carbohydrates (Wang, Chang, Inbaraj, & Chen, 2010) among which the water-soluble polysaccharide fraction has received a great attention in the last few years. In addition, arabinogalactan proteins have been identified as significant bioactive molecules for their hypoglycaemic and hypolipidaemic effect (Cheng et al., 2015; Masci et al., 2018).

The GBs protective and antioxidant role, evaluated by Oxygen Radical Absorbance Capacity (ORAC) and by radical scavenging activity, was correlated with the high content of phenylpropanoids and

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(iso)flavonoids (caffeic and chlorogenic acid, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside), coumarins, lignans (Donno, Beccaro, Mellano, Cerutti, & Bounous, 2015; Mocan et al., 2018; Zhang, Chen, Zhao, & Xi, 2016; Zhou et al., 2017).

A significant carotenoid fraction (CAR), responsible for the characteristic orange-red colour, makes GBs one of the richest CAR natural sources. Zeaxanthin dipalmitate (ZEA), molecule with a highly valuable biological role was the most representative compound of this class (Amagase & Farnsworth, 2011).

ZEA accumulates in the macula densa of retina playing a protective role, preventing ultraviolet radiation degenerative effects. Weller and Breithaupt (2003) reported lutein and zeaxanthin as protective agents towards age-related macular degeneration (AMD) showing dried wolfberries as a rich source of zeaxanthin esters. AMD is considered as a neurodegenerative disease that represents the leading cause of acquired blindness. This progressive illness affects a significant number of elder people (over 55 years, about 9% worldwide) and has a multifactorial aetiology. Genetic and environmental risk factors play an important role, but also smoking and dietary habits (Cascella et al., 2014; Sajilata, Singhal, & Kamat, 2008). Regular and abundant daily intake of fruits and vegetables, rich sources of carotenoid antioxidant pigments and many other bioactive molecules, has been associated with a reduced risk of chronic and degenerative diseases (Desmarchelier & Borel, 2017). The authors claimed that a number of factors could influence the bioavailability of the listed components, such as their chemical nature, food matrix, human metabolism, absorption efficiency in the intestinal lumen, and consequently their real efficacy in the health promotion.

The interest for GBs as food supplement is emphasized by the fact that different bioactive molecules, specifically hydrophilic polysaccharides and lipophilic carotenoids, exert different functions such as generic protection towards oxidation, type-2 diabetes, inflammation, cancer, that could converge in the prevention of specific illnesses. Type-2 diabetes, together with cardiovascular disease, could also have some key roles in AMD progression and diseases associated with retinopathies. The combined action of the hypoglycaemic polysaccharides that prevent the onset and the progression of diabetes (Masci et al., 2018) and of ZEA, active in the macular protection, could provide a synergic effect in the blindness prevention.

In the above discussed context, the aim of the present work was to monitor the carotenoid content in Goji berries cultivated in Italy, evaluating the differences among varieties, harvesting periods, seasons, and extraction procedures. CIELAB colorimetric and quali-quantitative HPLC-DAD analyses were performed and the obtained data were statistically analysed to build correlation models aimed to predict CAR and ZEA contents directly from colorimetric measurements.

2. Materials and methods

2.1. Materials

Ethanol (≥96%), double-distilled water, cyclohexane RPE, methanol RS and acetone RS for HPLC were purchased from Carlo Erba (Milan, Italy). HPLC-grade glacial acetic acid and ethyl acetate were obtained from Fluka (Milan, Italy). GBs were generously gifted by Azienda Natural Goji ® and were harvested at different commercial harvesting periods **1–5** (2015: July 6th **1**; July 23rd, **2**; August 3rd, **3**; and 2016: July 26th, **4**; August 4th, **5**) in Fondi (Latina province, Lazio region, Italy) based on their stage maturity as determined by the producer. They were "Poland" and "Wild" varieties, **P** and **W**, respectively. Ten different samples (**P1–P5** and **W1–W5**) were then collected, quickly frozen at −80 °C and stored at −18 °C, until the analyses were performed. Zeaxanthin dipalmitate standard (purity ≥ 98%) was purchased from Extrasynthese (Lyon, France).

Table 1
P1D–P5D, **P1U–P5U**, **W1D–W5D** and **W1U–W5U** ZEA, CAR and ORG yields from GB extractions. Ratio between ZEA and CAR is also reported. All data are expressed as mg per gram (g) of GBs dry weight. Sample names were compiled merging the selection **P** or **W**, the number of harvesting (**1–5**) and the homogenization technique (**D** or **U**) as reported in the text. Mean values were from four different experiments (errors in the range of 5–10% of the reported values).

SAMPLE	ORG	ZEA	CAR	ZEA/CAR ratio (%)
P1D	20.85	5.03	6.40	78.6
P2D	18.56	2.63	3.92	67.3
P3D	21.13	1.90	2.61	73.1
P4D	20.17	4.49	6.00	74.9
P5D	14.50	2.74	1.82	72.8
P1U	29.66	4.02	5.04	79.7
P2U	36.83	2.28	5.94	77.3
P3U	33.91	2.45	3.32	73.8
P4U	30.53	4.30	5.64	76.3
P5U	21.89	2.94	3.95	74.3
W1D	20.27	5.54	6.86	80.7
W2D	20.36	3.25	5.66	57.7
W3D	18.64	2.16	3.93	55.1
W4D	20.18	4.99	6.38	78.3
W5D	13.81	2.54	1.83	69.2
W1U	30.51	4.26	5.87	72.8
W2U	43.61	2.56	9.18	56.2
W3U	30.70	1.92	6.41	30.1
W4U	25.36	3.94	4.94	79.7
W5U	20.00	2.27	3.26	69.8

2.2. Sample preparation

The defrosted GBs were washed and wiped up on paper towel at room temperature. Then, they were ground and homogenized with two different procedures: at room temperature for 2 min by means of a domestic mixer at 16,000 rpm (**D** samples) or by a T18 Ultraturrax® homogenizer (IKA®, Staufen, Germany) at 10,000 rpm (**U** samples) Due to GBs lability, the procedure steps were carefully performed to reduce loss of pigments. The resulting fruit purees were further divided into two aliquots: one for the CIELAB colorimetric analysis and the second for the extraction procedure leading to a panel of 20 experiments (**P1D–P5D**, **P1U–P5U**, **W1D–W5D** and **W1U–W5U** – Tables 1 and 2).

Table 2
Colorimetric data of the Goji berry **P1D–P5D**, **P1U–P5U**, **W1D–W5D** and **W1U–W5U** samples. Mean values were from four different experiments (errors in the range of 1–2% of the reported values).

SAMPLE	L^*	a^*	b^*	C^*	h_{ab}
P1D	37.63	21.78	18.92	28.85	40.98
P2D	40.47	24.66	23.89	34.33	44.10
P3D	40.27	24.54	23.43	33.93	43.66
P4D	39.61	26.17	20.88	33.48	38.58
P5D	37.93	22.50	18.50	29.13	39.42
P1U	40.54	26.26	23.27	35.01	41.65
P2U	39.18	22.58	21.43	31.13	43.51
P3U	42.18	26.90	26.86	38.01	44.95
P4U	39.03	25.90	20.82	33.23	38.79
P5U	38.86	24.30	19.85	31.38	39.25
W1D	38.22	22.06	19.34	29.34	41.25
W2D	39.71	24.04	22.00	32.59	42.46
W3D	40.42	23.58	23.31	33.16	44.67
W4D	38.34	23.89	19.38	30.76	39.04
W5D	35.65	16.81	14.71	22.34	41.17
W1U	44.44	30.54	28.95	42.08	43.47
W2U	43.86	29.20	29.16	41.27	44.96
W3U	43.45	27.27	28.12	39.17	45.87
W4U	39.46	26.49	20.79	33.65	38.16
W5U	39.09	23.67	19.77	30.85	39.87