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Screening of Bioactivity in Extracts from Different Varieties of Lettuce †

Filomena Monica Vella [1], Roberto Calandrelli [1], Domenico Cautela [2] and Bruna Laratta [1,*]

[1] National Research Council (CNR), Institute of Research on Terrestrial Ecosystems (IRET), via P. Castellino, 111, 80131 Naples, Italy; filomenamonica.vella@cnr.it (F.M.V.); roberto.calandrelli@cnr.it (R.C.)

[2] Experimental Station for the Industry of the Essential Oils and Citrus Products (SSEA)—Special Agency of the Chamber of Commerce of Reggio Calabria, via T. Campanella, 12, 89125 Reggio Calabria, Italy; dcautela@ssea.it

* Correspondence: bruna.laratta@cnr.it

† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: Lettuce (*Lactuca sativa* L.) belongs to Asteraceae family and is a widely grown and a popularly consumed vegetable worldwide cause leafy vegetables are common items in a well-balanced diet. Lettuce is low in calories, fat and sodium. It is a good source of fiber, iron, folate, vitamin C and various bioactive compounds. Lettuce is consumed in salad mixes and, as its consumption is increasing, is becoming one of most 'healthy' food. In vitro and in vivo studies have shown anti-inflammatory, cholesterol-lowering, anti-diabetic, and antioxidant activities attributed to the bioactive compounds in lettuce. The healthy properties are attributed to a large supply of antioxidant compounds mainly polyphenols. As they act as free radical scavengers, both these secondary plant metabolites are fundamental to counter oxidative stress, inflammation, cancer, diabetes, age-related neurodegeneration and cardiovascular disease. Further, various studies have investigated the effects of the polyphenolic compounds present in green lettuce, in reducing oxidative and anti-inflammatory stresses. This work aims to assess polyphenolic content, as well as related antioxidant capacity of 16 lettuce types, belonging to butterhead (L. *capitata*) and crisphead (L. crispa) varieties, in order to know their potential correlation between phenolic content and antioxidant activity. Results have shown lattuce an interesting and cheap source of antioxidant phenolics as alternative natural compounds useful to retard oxidation in different food systems.

Keywords: lettuce; lactuca sativa; polyphenols; antioxidant activity

1. Introduction

Several pieces of scientific evidence promote a balanced, health-promoting type of diet, based on vegetables and fruit, as the basis to prevent the starting of chronic and age-related diseases [1–4].

The beneficial effects of fruits and vegetables are believed due to the presence of biomecules with healthy properties such as polyphenols that, with their antioxidant activity, play an important role in counteing oxidative stress and in protecting human body by related demage such as, inflammation, cancer, diabetes, age-related neurodegeneration, and cardiovascular diseases [5–8].

Among vegetables, lettuce (*Lactuca sativa* L.), which belong to the Asteraceae family, is one of the most widely grown and popularly consumed worldwide. The largest producer in the world is China, followed by the USA and Europe, that contribute about 57%, 14% and 11% of the total lettuce production, respectively [9]. In 2018, Italy was responsible of production of about 770,000 ton, which represented the 26% of European lettuce production [9].

Lettuce comes in a array of colors, sizes, and shapes and because of this variability it can be grouped by their types. According to Mou classification [10], there are six main lettuce groups based upon leaf shape, size, texture, head formation, and stem type: crisphead (var. *crispa*), butterhead (var. *capitata*), romaine or cos (var. *longifolia*), leaf or cutting (var. *acephala*), stem or stalk (var. *angustana*), and Latin lettuce (no scientific name).

Lettuce is popularly consumed as fresh product in salad mixes and nowadays consumption of salads is increasing, above all for to the consumer perception of healthy food [11]. Moreover, since lettuce is generally eaten raw, more nutrients and bioactive compounds are retained compared to other vegetables that are cooked or processed, avoiding in this way the degradation of the thermolabile phytochemicals with potential nutraceutical interest [8,12]. Various studies have investigated the effects of the polyphenolic compounds present in lettuce, in reducing oxidative and anti-inflammatory stresses [11–13].

The objective of this study was to provide information about the potential use of lettuce as an effective source of natural antioxidants occurring naturally in foods; specifically 16 lettuce, belonging to butterhead (var. *capitata*) and crisphead (var. *crispa*) types were compare with respect to its contents of polyphenols and antioxidant activity, with the aim to determine the lettuce type that provides the greatest health-beneficial bioactive compounds.

2. Experiments

2.1. Reagents and Standards

All reagents and solvents were of analytical grade or otherwise stated. Gallic acid standard and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant Material and Sample Extraction

In this study 16 lettuce, belonging to butterhead (var. capitata) and crisphead (var. crispa) varieties were hand-harvested in Villa Literno (Caserta province, Italy; 41°0′33 N–14°4′34 E) at commercial maturity condition.

For each plant, outer leaves (second stage of leaves) were detached and lightly washed with distilled water to remove eventual residues. Five grams of leaf were placed in an extracting solution of 49 mL methanol and 1 mL HCl 37% [12]. The solution was covered to avoid evaporation, shaken at 100 rpm at 20 °C in the dark for 2 h. The resulting extracts were filtered through Whatman paper filter, dried using a rotary evaporator (IKA RV8) and immediately stored at −20 °C, until analyses were performed.

2.3. Total Polyphenols Content

The total phenolics content was determined by the Folin-Ciocalteu assay [14]. Briefly, different aliquots of extracts were diluted to a 150 µL final volume with distilled deionized water. Folin-Ciocalteu's reagent (750 µL) and 600 µL of 7.5% Na_2CO_3 were added to the sample. The absorbance at 765 nm was read after incubation in the dark for 2 h at room temperature. Total phenolics content was expressed as mg of gallic acid equivalents (GAE) per g of fresh weight (FW). Samples were analysed in triplicate.

2.4. In Vitro Antioxidant Activity

The antioxidant activity of lettuce extracts was evaluated the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according Blois procedure [15]. Briefly, 1.35 mL of 60 µM DPPH radical in methanol were added to different extract concentrations, ranging from 10 to 1000 µg/mL. The decrease in absorbance at 517 nm was continuously determined until absorbance stabilization. The radical scavenging activity percentage (%RSA) of DPPH discoloration was calculated according to the formula:

$$\%RSA = ((A\_DPPH-A\_s))/A\_DPPH \times 100 \quad (1)$$

where AS was the absorbance of the solution when the essential oil was added and ADPPH was the absorbance of the DPPH solution. The extract concentration (EC) necessary to achieve a 50% of radical DPPH inhibition (EC50) was obtained by plotting the RSA percentage as function of extract concentrations and was expressed as mg/mL.

2.5. Statistical Analyses

Samples were analyzed in triplicates and all results were expressed as mean ± standard deviation (SD). Means, SD, calibration curves and linear regression analyses (R2) were determined using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Correlation analyses were carried out by using CORREL function in Microsoft Excel 2013. Pearson correlation coefficients (r) were calculated and followed by t-Student test, with two-sample equal variance and two-tailed distribution. Differences at $p < 0.05$ were considered significant and $p < 0.01$ were highly significant.

3. Results and Discussion

Phenolic compounds refer to an important group of plant secondary metabolites responsible for the plant's defense system. More than 8000 polyphenols have been identified in all plant organs [7] and affect the sensory and nutritional properties of plant-based foods [16]. Polyphenols have been described to have greater antioxidant activity than vitamins C and E [17]. Individual phenolic compounds and vegetables that contain phenolic compounds have shown beneficial effects against oxidative stress, inflammation, cancer, diabetes, age-related neurodegeneration, and cardiovascular diseases [5–8].

In this study 8 butterhead (code number 6, 7, 10, 11, 12, 13, 14, and 15) and 8 crisphead (code number 16, 17, 18, 19, 20, 21, 22, and 23) lettuces were analyzed considering both total polyphenols content and antioxidant activity. The results were reported in Table 1 and in Figure 1, respectively.

Total phenolic content in lettuce varies among lettuce types. In general, crisphead lettuces were on average lower in total phenolic content compared to butterhead types. Butterhead number 22 had the highest value of total polyphenols, as shown in Table 1.

Table 1. Poyphenols content (mg GAE/100 g FW) of lettuces.

Lactuca sativa Type	Code Number	mg GAE/100 g FW *
crisphead	6	109.69 ± 7.12
crisphead	7	100.34 ± 6.83
crisphead	10	72.11 ± 3.75
crisphead	11	80.44 ± 3.31
crisphead	12	121.66 ± 5.38
crisphead	13	90.91 ± 5.59
crisphead	14	119.40 ± 7.58
crisphead	15	58.67 ± 2.57
butterhead	16	85.51 ± 1.82
butterhead	17	95.36 ± 1.97
butterhead	18	99.88 ± 2.44
butterhead	19	118.67 ± 7.54
butterhead	20	79.00 ± 4.27
butterhead	21	101.80 ± 5.29
butterhead	22	142.70 ± 6.97
butterhead	23	123.54 ± 8.40

* GAE = gallic acid equivalent; FW = fresh weigth.

Differences in phenolic content of lettuce types could be due to genetic, agronomical and environmental factors that influence the chemical composition and in turn affect polyphenolic amount [11]. Particularly, the abundance of these compounds may be altered by many factors, such as light intensity (quality and quantity), water availability and irrigation regime, nutrient supply, use of pesticide, weather conditions, growing season as well as fertilization, production, cultivation, and storage procedures (Durazzo et al., 2014, Kim et al., 2016).

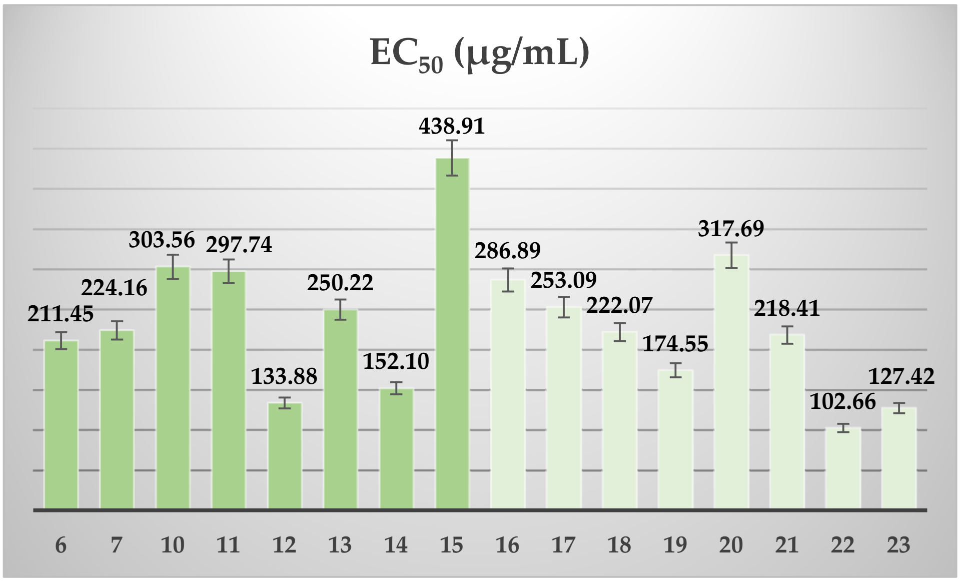

Figure 1. Antioxidant activity of lettuces expressed as EC50 (µg/mL). Green = crisphead; light green = butterhead.

The results of DPPH radical scavenging activity was presented as EC50 values. As showed in Figure 1, butterhead number 22 possessed the lowest EC50. Moreover, a highly significant negative correlation ($p < 0.01$) existed between total polyphenols and antioxidant activity of lettuce extracts. As a low EC50 indicates a high antioxidant activity of a sample, this result means that an increase in bioactive compounds corresponded to a lower extract concentration necessary to achieve 50% of DPPH inhibition, thus suggesting a higher antioxidant activity.

The DPPH radical scavenging activity is greatly influenced by the total phenolic content of the lettuce samples. In particular, the variation in polyphenolic content causes a variation in DPPH radical scavenging of extracts of lettuce. As mentioned earlier, different geographical locations, environmental factors and postharverst handling could affect the total phenolic content that indirectly altered the antioxidant activity of the lettuce samples [8,18].

4. Conclusions

Lettuce is high in water content (95%) and low in calories, is characterized by dietary fiber, presence of several important dietary minerals (low amount of Na and a relatively good amount of Fe), and various vitamins (folate and vitamins C).

Despite consumer expectations, lettuce can provide considerable amounts of healthy nutrients, particularly phenolic compounds. The results of this study demostrate that lettuce is rich in bioactive phytochemicals, thus increasing the nutritional value of lettuce in human diet. Notably, butterhead types were found to be slightly richer in polyphenols than crisp lettuce, hence recommending more the use of the former variety.

Further studies concerning the bioavailability of polyphenols in lettuce and their nutraceutical potential application are needed to evaluate the health-promoting benefits.

Author Contributions: Conceptualization, B.L. and F.M.V.; investigation, R.C., D.C., and F.M.V.; data analysis D.C., B.L. and F.M.V; writing-review and editing B.L. and F.M.V. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors are grateful to Agostino Navarro for their helpful assistance during the sample preparation.

Conflicts of Interest: the authors declare no conflict of interest.

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