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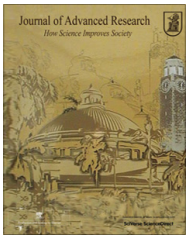

ORIGINAL ARTICLE

The effects of different night-time temperatures and cultivation durations on the polyphenolic contents of lettuce: Application of principal component analysis

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ABSTRACT

The present study was conducted to characterize the polyphenolic contents of lettuce leaves grown under different night-time temperatures (4, 12, and 20 °C) and cultivation durations (5, 15, and 20 days) using high performance liquid chromatography-tandem mass spectrometry

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(LC/MS/MS). The assay method was validated based on specificity, linearity, accuracy, precision, and the performance limit. The total polyphenolic contents were highest (2462.6 mg/kg) after transplantation at a night temperature of 20 °C on day 20 and lowest (1132.7 mg/kg) at the same temperature on day 5. Quantification and principal component analysis showed that the relative contents of quercetin and kaempferol were markedly higher during the early stage of cultivation (day 5) than those of day 15 and 20, and that night-time temperatures of 12 and 20 °C on day 20 were favorable for producing polyphenol-rich lettuce containing caffeic acid. In conclusion, a synergistic effect between high night-time temperatures (12 and 20 °C) and cultivation duration (20 days) produced lettuce rich in polyphenols compared to that at low temperature (4 °C).

Introduction

Lettuce (*Lactuca sativa* L.), a leafy vegetable native to the Mediterranean area, was cultivated in Egypt as early as 4500 BC [1]. It belongs to the Compositae family (Asteraceae) with a high rank both in production and economic value among vegetables grown in the Republic of Korea [2]. Lettuce is conventionally consumed in salads, and its seeds are utilized in folk medicine for treating rhinitis, asthma, cough, insomnia, and pertussis [3]. Lettuce contains multiple health-beneficial components, including polyphenols, ascorbic acid, carotenoids, and tocopherols. These compounds have protective effects against cancers, cardiovascular disorders, and other chronic diseases [4].

Polyphenols possess powerful antioxidant activities and protect animal cells from the harmful effects of reactive oxygen species (ROS), which are produced from a wide range of stressors [1]. Polyphenolic contents vary considerably among plants, depending on the type and intensity of the stressors during their growth and management [5] In this context, phenylalanine ammonia-lyase (PAL), a key plant enzyme in the biosynthesis of various polyphenols, is activated via a number of biotic and abiotic stressors, including radiation, temperature, plant hormones, wound, and disease [6–8]. Induction of this enzyme increases the production of phenolic compounds, including tannic, gallic, caffeic, chlorogenic, and cinnamic acids in lettuce grown under low temperature [5,9]. The PAL enzyme is significantly correlated with temperature in plants, and its activity increases in response to either low or high temperature [10]. Lower temperatures decrease fresh lettuce weight [11,12], whereas higher temperatures induce bolting [13]. This means that quality and productivity are not guaranteed under stressful temperatures.

Lettuce is usually cultivated under outdoor conditions with day and night-time temperatures of 17–22 °C and 3–12 °C, respectively [11]. Under controlled greenhouse conditions, the optimum night temperature is 15–20 °C, as suggested by Choi and Lee [12]. The night-time temperature has additional importance, as heating and cooling in winter and summer add an extra cost to greenhouse maintenance. However, to the best of our knowledge, there have been no reports on the role of night growth temperatures and cultivation durations on polyphenols in leaf lettuce production.

In the present study, polyphenols were determined and profiled in lettuce leaves in response to variations in growth conditions, including night-time temperatures and the duration of greenhouse cultivation using liquid chromatography-tandem mass spectrometry (LC/MS/MS) and principal component analysis (PCA). Polyphenol characterization utilizing LC/MS/MS is advantageous because it does not require extensive purification steps. LC/MS/MS is a powerful tool that provides clear and characteristic fragment patterns to identify plant polyphenols [14]. Our results will be useful to develop cultivation guidelines for the production of health-beneficial polyphenol-rich lettuce.

Material and methods

Materials and chemicals

Lettuce (*L. sativa* L., cv Cheongchima) seeds were germinated in plug-cell trays filled with 'Tosilee' (Shinan Grow Co., Jinju, Republic of Korea) commercial media on May 10, 2011. After four leaves were opened, they were transplanted to 9 cm plastic pots and cultivated in three glass chambers (KGC-175 V, Koencon, Hanam, Republic of Korea) with a day temperature of 22 °C and night temperatures of 4, 12, and 20 °C, until harvest. The photoperiod was 12-h light/12-h dark and was provided by fluorescent lamps (approximately 450 μmol m^{-2} s^{-1}). Relative air humidity was approximately 65%. Water was supplied daily via overhead irrigation, and nutrient solution (Hoagland, pH = 5.9 ± 0.2, EC = 1.2 mS cm^{-1}) was provided every 4 days. The plant density was 36 plant/m^2 in each treatment. The plants were rearranged every 3 days to minimize position and/or edge effects in glass chamber. The leaves were washed with distilled water, lyophilized, and stored in dark glass containers at –20 °C pending analysis.

Caffeic acid, kaempferol, and quercetin were used as external standards after recrystallization in ethanol (Sigma–Aldrich Co., St. Louis, MO, USA). The purity of all standards was confirmed by HPLC to be at least 99%. All solvents and water were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Republic of Korea).

Extraction and purification

Lyophilized leaves (0.5 g) were ground into a powder and poured into 25 mL of aqueous 80% methanol. The mixture was homogenized using a Polytron blender (Brinkman Instruments, Westbury, NY, USA) for 5 min at room temperature and treated in a sonicator (100 W, 42 K*HZ*, Bransonic 3510R-DTH, Danbury, CT, USA) for 10 min. The extract was filtered through a glass filter under reduced pressure and centrifuged at 4000*g* (SCT4B centrifuge, Hitachi, Ibaraki, Japan). The supernatant was filtered through a PTFE syringe filter (Titan, 0.45 μm, SMI−Lab Hut Co., Ltd. Maisemore, UK), and the filtrate was stored at −20 °C until analysis.

LC/MS/MS

The LC/MS/MS experiment was performed according to our previously reported methodology [15] with the exception of