

Extraction and Free Radical-scavenging Activity of Phenols from Fresh-cut Lotus Roots during Browning

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Abstract: Phenols were extracted from fresh-cut lotus roots during storage. Phenolic content and free radical-scavenging capacity were studied. The phenols in fresh-cut lotus roots identified by HPLC were included gallic acid, hydroxyltyramine, catechin and caffeic acid. The phenolic content in fresh-cut lotus roots presented a decreasing trend during storage, while the free radical-scavenging capacity per unit mass revealed an ascending-descending-ascending trend. Meanwhile, a close relationship between phenolic content and composition and free radical-scavenging capacity was observed. The phenolic composition in fresh-cut lotus roots remained unchanged during storage at room temperature. Pyrogallol acid, catechol and caffeic acid were closely correlated with browning, especially the amount of pyrogallol acid.

Key words: fresh-cut; lotus root; phenols; free radicals; browning

鲜切莲藕褐变期间酚类提取及其对自由基的清除能力

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摘要: 从不同贮藏时间的鲜切莲藕中提取出酚类物质, 经高效液相色谱法(HPLC)对其进行定性和定量分析, 并对其清除自由基能力进行比较。结果表明: 藕片中主要含有焦性没食子酸、羟酪胺、儿茶酚和咖啡酸4种。鲜切莲藕贮藏过程中总酚含量呈下降趋势, 单位质量酚清除自由基能力则呈现先升后降最后略有上升的趋势。说明酚类物质含量的不同与其抗氧化能力存在密切联系。常温贮藏期间, 鲜切藕片中的酚类物质种类不变, 但含量会改变, 与莲藕褐变关系密切的是焦性没食子酸、儿茶酚和咖啡酸, 其中焦性没食子酸与莲藕清除自由基能力关系密切。

关键词: 鲜切; 莲藕; 酚类; 自由基; 褐变

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Lotus root (*Nelumbo nucifera gaertn*), which has been recognized as a medical-food plant, is a multi-purpose aquatic economic crop in China^[1-2]. Because of its crisp and tender taste, lotus root is suitable for fresh-cut use. As a newly arisen product, fresh-cut lotus root is popular in the national supermarkets^[3]. However, lotus root is a type of vegetables with high moisture content and a thin skin. Therefore, the lotus root has white rind and flesh which turns brown easily during storage and processing. Fresh-cut processing results in tissue damage, and stimulates oxidation of cellular components which causes tissue browning^[4-5],

leading to change in flavor, reduction in nutritional quality and a decrease in consumer acceptance. These are the main limitations in the shelf-life and marketability of fresh-cut lotus root.

It has been proved that there is certain relationship between the browning in fruits and vegetables and the accumulation of quinone, which is the oxidation product of phenols in plant tissue^[6-7]. At locations where browning occurred easily, the phenolic content was also found to be high. Phenols form one of the main classes of secondary metabolites with a large range of structures and functions, but

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this definition is not entirely satisfactory. Based on metabolic origin, plant phenols are regarded as those substances derived from the shikimate pathway and phenylpropanoid metabolism^[8]. The mechanical damage, caused by fresh-cutting activated the key enzymes of shikimate pathway and phenylpropanoid metabolism, resulting in synthesis of lots of phenols in the tissue^[9]. There is also a close relationship between the phenolic content and the browning degree of fresh-cut burdock^[10]. Most of the phenols, therefore, can be recognized as “potential unstable factors” in fruits and vegetables^[11].

Different kinds of fruits and vegetables contain different types of phenols which serve as browning substrates. High contents of pyrocatechol compounds, a class of phenols, were identified after HPLC analysis. Hence, pyrocatechol compounds can serve as one of the main substrates in plant browning^[12]. For example, Lan Bifeng et al.^[13] reported that catechol is the main enzymatic browning substrate and results in the formation of brown pigments of lotus root tissue. The main substrate causing enzymatic browning in yam was chlorogenic acid^[14]. This was simply due to the oxidation of phenol hydroxyl group, especially ortho-phenol hydroxyl group on catechol or pyrogallol. Under suitable conditions, phenol hydroxyl group was quickly oxidated to the brown product quinone.

As phenols are abundant in variety, their role in enzymatic browning is quite complicated. Some species of phenols can exist as phenolic substrates, which promote the occurrence of browning. Others have a strong capacity of reactive oxygen, which can combine with some free radicals produced by the lipid peroxidation and reduce or prevent the oxidation process in tissue, resulting in phenols with strong antioxidant and free radical scavenging ability^[15]. Phenols, therefore, mainly provide the antioxidant activities of plants and these two factors are directly related^[16].

The earliest quantitative determination of phenols was by paper-chromatographic method^[17]. Currently analytical techniques applied for the determination of phenols are mainly high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), ultraviolet detection (UV), fluorescence detection, electrochemical detection or mass spectroscopy (MS)^[18-19].

It has shown that enzymatic browning is the main reason for browning in lotus root^[20]. Being the substrates for enzymatic browning in fresh-cut lotus root, phenolic compounds directly affect the speed and time of browning

reaction. Thus, the main reason for browning of lotus root is phenolic oxidation. Previous studies about phenols in lotus root focused on extraction technology^[21], antimicrobial activity^[22], antioxidant activity^[23], etc. However, few studies have been done on qualitative analysis of phenolic compounds in fresh-cut lotus root during browning development. The aim of this study, therefore, is to investigate the changes in phenolic compounds involved in browning during storage and for a further understanding of the browning process in lotus root. This would be important in explaining the browning mechanism and helpful in the prevention of browning in lotus roots.

1 Materials and Methods

1.1 Materials, reagents and instruments

Lotus roots (cv. 3735) were collected from Jiangsu province, China. The sturdy lotus roots of moderate maturity, with good appearance and non-mechanical wounding, were dipped in running water and scrubbed. After pre-cooling at 4 °C for 24 h, the selected lotus roots were peeled and cut into slices of 0.3—0.5 cm thickness. The root slices were packaged in high density polypropylene (HDPE, thickness: 15 μm; size: 25 cm×38 cm) plastic bags and then stored at room temperature until the slices were fully brown. Samples, each 500 g in weight were taken on a daily basis. All tests were repeated 3 times.

Chlorogenic acid (Sigma, USA); catechin, epicatechin (Fluka, Switzerland); caffeic acid (Acros, Belgium); other chemicals were HPLC reagent.

Agilent 1210 high performance liquid chromatograph (Agilent, USA).

1.2 Methods

1.2.1 Phenolic content

Phenols were extracted according to the method by Yan Shoulei et al.^[24] with some modifications. Samples each 20 g were extracted in cold 60% ethanol for 6 h and the extraction solution made up to a volume of 100 mL. After centrifuging at 12000 r/min for 20 min, phenolic content was determined by the methods of Pirie et al.^[25] with some modifications. 5.0 mL of 1% HCl-ethanol was added to 2.0 mL of the phenolic extracts. After centrifugation for 20 min at 12000 r/min, the absorbance of the supernatant was determined at 280 nm. Total phenols (TP) contents were calculated as gallic acid equivalents per gram of fresh weight, using a standard curve^[26] generated with 50 — 500 μg range.

1.2.2 Browning degree assessment

Browning was assessed by measuring the color change of the total browned areas on each sample^[27]. CIE L^* parameter was measured with a portable tristimulus colorimeter (WSC-S, Shanghai). Samples of 10 pieces each were evaluated per each treatment at each sampling time.

1.2.3 Free radical scavenging activity

The radical generation system model was assayed by modification of the Fenton reaction method by Yan Jun et al.^[28]. The reaction solution contained 0.3 mL of 0.02 mol/L ferrous sulfate and 0.25 mL of 0.02 mol/L hydrogen peroxide and was incubated in a 10 mL graduated tube at 37 °C for 30 min. After mixing, 1.0 mL of 0.01 mol/L salicylic acid and 1.0 mL phenolic extract was added to each tube. Ultra pure water was used to make up the final volume to 10.0 mL. Absorbance was recorded at 510 nm. The ability of scavenging $\cdot\text{OH}$ was expressed as clearance rate and was calculated as follows:

$$\text{Clearance rate/\%} = \frac{A_0 - A_x}{A_0} \times 100$$

Where: A is the absorbance; A_0 represents clearance in the presence of ultra pure water; A_x represents clearance in the presence of ultra pure water and sample extract.

1.2.4 UV-scan analysis

Phenolic extraction was carried out by UV-Vis spectrophotometer at a wavelength ranging from 200 nm to 400 nm; and methanol was taken as the scan baseline. The standard samples were dissolved in methanol (0.1 mmol/L) and the operation steps were the same as above.

1.2.5 HPLC analysis

The mobile phase was acetonitrile-water (ACN- H_2O , 30%) containing 1.0% acetic acid. The extractions were diluted with mobile phase in a ratio of 1:5 and centrifuged at 12000 r/min for 20 min. The pure supernatant was filtered through a 0.45 μm PVDF membrane filter into HPLC vials and injected for analysis. Phenolic compounds analysis was conducted according to Wang Qingzhang et al.^[29] with some modifications. The separation was performed on an Agilent Zorbax SB-C₁₈ (4.6 mm \times 250 mm, 5 μm) column at a flow rate of 1.0 mL/min. The UV detection wavelength was 280 nm and SB-C₁₈ temperature was 30 °C.

1.3 Statistical analysis

Each experiment was taken three replicates. Data were subjected to analysis of variance using SPSS 18.0 software. Least significant differences (LSD) were calculated to compare significant effects at the 5% level.

2 Results and Analysis

2.1 Browning degree, phenolic content and $\cdot\text{OH}$ scavenging activity

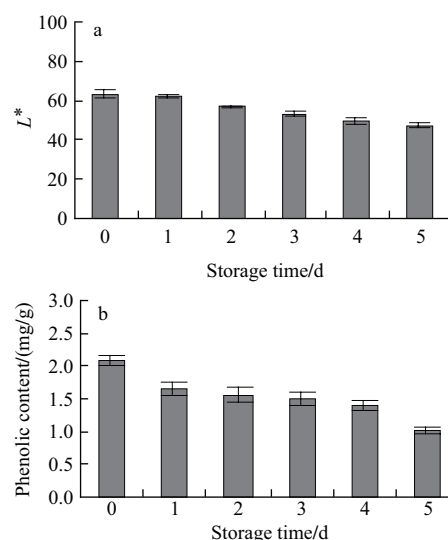
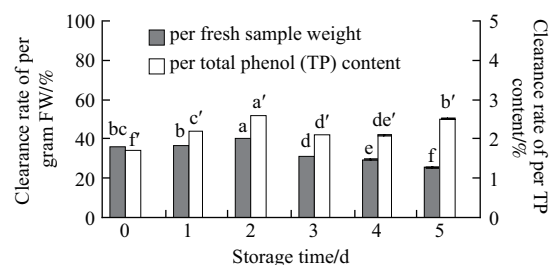


Fig.1 Change in L^* and phenolic content of fresh-cut lotus roots during storage at room temperature

During storage at room temperature, the browning degree of fresh-cut lotus root became more serious. Storage period of untreated fresh-cut lotus root at room temperature did not exceed 5 d. Roots had completely lost their traditional flavor and nutritive value by the last day of storage. Results for L^* and phenolic content are displayed in Fig.1. L^* value was the indirect representation of browning degree, as the former had a significant negative correlation to the latter. A decrease of 50% was observed in the phenolic content at the initial and late stages. The significant positive correlation was existed between phenolic content and L^* . That is, phenolic content decreased with the increase in the degree of browning. From another point of view, this is also proved that browning was related to the accumulation of oxidation of phenolic compounds and their products quinone in lotus root tissue.



FW. Fresh weight; Different letters in the same column mean significant at the 0.05 level. The same as in Fig.4.

Fig.2 Change in free radical-scavenging capacity of fresh-cut lotus roots during storage at room temperature

The clearance rate of phenolic extraction in lotus root first increased and later decreased during storage. •OH scavenging activity per gram of fresh weight (FW) samples presented an up-down trend as illustrated in Fig.2. The maximum value observed was 40.09% on the second day and the minimum was 25.39% on the fifth day. It can therefore be inferred that the composition of phenolic in fresh-cut lotus root might change during storage. •OH scavenging activity per microgram of phenols presented an up-down-up trend; with the highest point being 2.58% on the second day and the lowest 1.72% in the initial stage. This result showed samples on the second and last day contained some specific phenols that had higher free radical scavenging capacity. The radical scavenging activities of most fruits and vegetables have some relevance with phenolic content. They were different because of diversity of phenolic components and varying quantities of effective phenols^[30]. There was positive correlation between TP contents and free radical scavenging capacity in fresh-cut lotus root, although it was not significant. Therefore, high phenolic content does not mean higher antioxidant activity. Many reports have shown that antioxidant activities in plants depend on phenolic species^[31].

2.2 The determination of pohenol components of fresh-cut lotus root during storage

UV scan of phenolic extract in fresh-cut lotus root has 2 peak absorptions. The initial peak absorption wavelength was 221 nm and the second maximum one was 273 nm. Phenolic extractions during storage had the same spectrum and maximum absorbance. There were only some slight differences in peak height. This paper did not list their UV spectrum because of only for qualitative analysis. It was, to some extent, concluded that phenolic compounds in lotus root might contain gallic acid, chlorogenic acid or caffeic acid (Table 1). Because the blue shift or red shift of the absorption peak would inevitably occur during scanning, the two peak widths were not sharp, hence it was not possible to precisely infer to the specific phenolic component.

Table 1 Ultraviolet spectral data and retention time for phenolic components

Standard sample	Ultraviolet absorption peak value /nm	Retention time /min
Gallic acid	221, 273	2.28±0.02
(+)-Catechin	228, 278	3.81±0.03
Hydroxytyramine	229, 283	3.25±0.05
Guaiaicol	228, 276	7.93±0.04
(-)-Catechin	223, 278	5.31±0.03
Pyrogalllic acid	236, 267	2.94±0.04
Caffeic acid	219, 244	8.93±0.03
Chlorogenic acid	219, 247, 303, 330	4.33±0.02

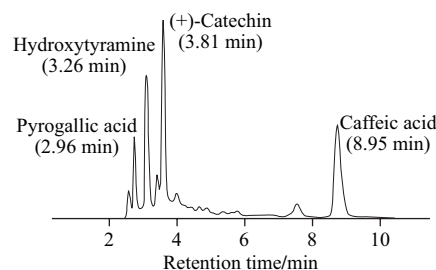


Fig.3 HPLC of fresh-cut lotus roots

HPLC of phenolic extractions in fresh-cut lotus root during storage is shown in Fig.3. There were 4 main absorption peaks, and retention times were 2.95, 3.26, 3.81 min and 8.95 min, respectively. Their similar peak shapes also indicates that the UV scanning spectrum was same. According to Table 1, fresh-cut lotus root contains 4 major phenolic components, including pyrogalllic acid, hydroxytyramine, (+)-catechin and caffeic acid.

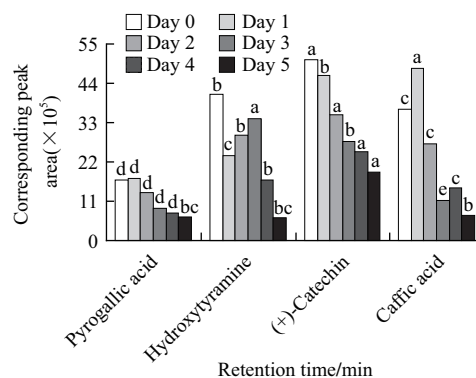
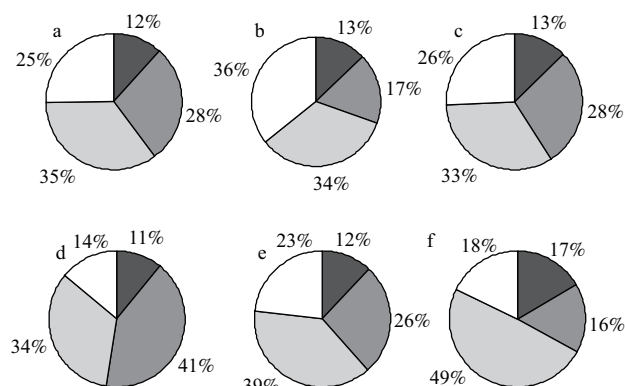


Fig.4 Different strong absorption peaks of fresh-cut lotus roots during storage at room temperature



■ Pyrogalllic acid; □ (+)-Catechin; ■ Hydroxytyramine; □ Caffeic acid;
a. Day 0; b. Day 1; c. Day 2; d. Day 3; e. Day 4; f. Day 5.

Fig.5 Different peak area ratios from strong absorption peaks of fresh-cut lotus roots during storage at room temperature

Phenolic components' relative contents were calculated on basis of peak area ratio in Fig.4. (+)-Catechin was the

major component in fresh-cut lotus root during storage; except at day 1 and day 3. Its content changed from 35% to 49% in Fig.5. On the second day, caffeic acid content was less more than (+)-catechin content and the relative content was 36%. On the fifth day, the relative content of hydroxytyramine was 41% and constituted the main component. (+)-Catechin content decreased during storage, and the trend was the same as the change in TP contents. Because the proportion of (+)-catechin in TP was more than 30%, its trend could reflect the change of TP contents. Pyrogalllic acid content increased a little on the first day and decreased afterwards. The change of hydroxytyramine content was complex. Its maximum value was on day 0, and then decreased with a fluctuation. The change of caffeic acid presented M-shape. The results showed that the phenols metabolism in fresh-cut lotus root during storage is a dynamic process, not simply a change in quantity.

Phenolic components did not change while change trends of their contents were different from phenolic content except (+)-catechin. This observation maybe attribute to the increased browning intensity. Enzymes had different optimum substances in enzymatic reactions, so that the change of each phenolic component was not the same. L^* had a significant positive correlation with (+)-catechin content, pyrogalllic acid content and caffeic acid content, and relative coefficient decreased one by one. There was no significant correlation between L^* and hydroxytyramine. Although fresh-cut lotus root browned easily under high phenolic content conditions, specific phenolic monomers had influence on the occurrence of browning. (+)-Catechin is also called catechol. Pyrogalllic acid is known as 1,2,3-trihydroxybenzene. Caffeic acid has a benzene ring structure on the adjacent hydroxide group. Hydroxytyramine, also known as dopamine, has an amino group. All of them are hydroxybenzoic acids, which in common have the C_6-C_1 structure; also are aromatic compound with a three-carbon side chain. However, catechol is the main substrate in lotus browning which is similar to the previous studies^[12]. (+)-Catechin made the greatest contribution to browning in lotus root, then pyrogalllic acid and the third was caffeic acid. The higher activity of hydroxytyramine could be due to the $CH=CH-COOH$ group which ensures greater H-donating ability and radical stabilization than $-COOH$ group^[32].

As far as we know, $\bullet OH$ is one of the most active and toxic oxygen free radicals^[33]. It reacts with almost any molecule in plant cells and mediates some biochemical

processes, such as lipid peroxidation, protein degradation, polymerization, nucleic acid cleavage, polysaccharide fracture and induces lesions of tissue cells, and thus accelerates aging^[34]. $\bullet OH$ plays an important role in browning of fresh-cut lotus root, and its accumulation is harmful to lotus root^[35]. Antioxidant activity is mainly provided by phenols in plants^[16]. The antioxidant properties of phenols are reflected through various ways. Phenols have scavenging activities because they have many phenolic hydroxyl as hydrogen donor. Phenols can reduce the highly active 1O_2 into a lowly active 3O_2 , which reduces the chances of free oxygen radicals being produced. Phenolic radicals with lower activities are also generated to prevent chain reaction by free radical oxidation. Most phenols have ortho-diphenol hydroxyl that chelates with metal ions and reduce their catalyst to oxidation. In addition, plant phenols have a synergistic effect with other antioxidants such as VC and VE. Therefore, antioxidation of plant phenols is a comprehensive effect^[15,22,36].

There was significant positive correlation between pyrogalllic acid content and clearance of $\bullet OH$ in fresh-cut lotus root. Not all of the phenolic components could play a role in scavenging free radicals. Only some specific polyphenols, such as pyrogalllic acid, could carry out this function. Compared to other phenols, pyrogalllic acid had the strongest antioxidant activity. The antioxidant activity in plants is mainly dependent on molecular structure^[37]. The dihydroxyl group is the main active antioxidant site, and its activity is ten times more than non-dihydroxyl phenolic compounds^[38]. Phenolic compounds form new substances with free radical by semi-quinodimethane. The structure of semi-quinodimethane is relatively stable and antioxidant activity is enhanced with increase of the dihydroxyl group. When the group accounts reach a certain number, the antioxidant capacity no longer increases^[39]. It was implied that pyrocatechol compound may contribute to tissue browning, with pyrocatechol compound initiating the oxidation and producing the color by polymerization^[40].

3 Conclusions

According to the UV-Vis and HPLC analyses, fresh-cut lotus roots mainly contain 4 types of phenolic components named pyrogalllic acid, hydroxytyramine, (+)-catechin and caffeic acid. By determining L^* value in fresh-cut lotus root stored at room temperature, browning degree displayed a decreasing trend. Meanwhile, TP contents also decreased

during storage. The free radical scavenging activity was first raised but decreased later. The effect of phenolic components on browning in fresh-cut lotus root can mainly be manifested in two aspects. On one hand, browning degree in lotus root is enchanted by oxidation of the major phenolic substrates, such as (+)-catechin, pyrogalllic acid and caffeic acid; on the other hand, pyrogalllic acid had a good ability to scavenge $\bullet\text{OH}$. The browning degree and ability of scavenging free radicals were related with phenolic contents and components of fresh-cut lotus root.

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