Crocins

Subjects: Neurosciences

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In the extraction of geniposide for the development of natural food colorants from the dried fruits of Gardenia jasminoides Rubiaceae, the gardenia fruit waste (GFW) still remaining 0.86% (*w*/*w*) of crocins has always been discarded without any further treatments. Crocins were extracted firstly by 50% of ethanol in the highest yield of 8.61 mg/g (*w*/*w*) from GFW. After the HPD-100 column fractionation in the collecting of crocins, the conversion ratio of 75% of crocins to crocetins can be obtained from the commercial available enzyme- Celluclast® 1.5 L. The crocins hydrolyzed products, were then separated through the HPD-100 resin adsorption and finally purified with the centrifugal partition chromatography (CPC) in single-step to obtain TC in a purity of 96.76 ± 0.17%. Conclusively, the effective enzyme transformation and purification co-operated with CPC technologies on crocins resulted in a high purity product of TC may be highly application in the commercial production.

crocins trans-crocetin enzyme transformation centrifugal partition chromatography

1. The Percent Extractability and Total Content of Crocins in the Dried Gardenia Fruit

Saffron is the main natural source of crocins (crocetin esters), which are water-soluble carotenoid derivatives constituting in a general content ranges between 16–28% in dried stigmas [1]. Because of its rarity and extremely high price of saffron, seeking alternative sources, i.e., the agricultural wastes have extremely high potential value. In this study, ethanol was selected as the solvent for its safety in human consumption and less negative environment impacts [2]. Ethanol at 25–95% was respectively used to extract the crocins from dried gardenia fruit (GF) and gardenia fruit waste (GFW). The optimum ethanol concentration for extraction of crocins from the intact dried gardenia fruit powder was determined to be 50% with yield of 25.63 ± 2.73%, and the total content of crocins was 14.09 ± 1.02 mg/g (**Table 1**). Thus, the 50% ethanol was applied to the extraction of crocins in the GFW, which has been extracted out geniposide and crocins, the raw material of colorant production, by using the solvent extraction of 40% ethanol. Interestingly, there was 8.61 mg/g of crocins still remained in the GFW. The effect of ethanol on the extraction efficiency of crocins was similar to that the extraction using Liquid CO_2 under the ethanol modifier addition of 50 or 80% ethanol [3]. It was speculated that the moderate alcohol % used in the extraction of geniposide might incapable in the total extraction of crocins.

Table 1. The extractability and total content of crocins from the dried gardenia fruit (GF) and gardenia fruit waste (GFW).

Ethanol (%)	Yield of Extract (*w*/*w* %) [1] GF	GFW	Total Content of Crocins (mg/g DW) [1] GF	GFW
25	19.90 ± 1.83 [b]	10.28 ± 0.99 [b]	10.28 ± 0.99 [b]	4.15 ± 0.52 [b]
50	25.63 ± 2.73 [a]	14.09 ± 1.02 [a]	14.09 ± 1.02 [a]	8.61 ± 0.63 [a]
75	14.26 ± 0.41 [c]	9.20 ± 0.34 [b]	9.20 ± 0.34 [b]	6.13 ± 0.41 [a]
95	10.77 ± 0.98 [c]	4.09 ± 0.30 [c]	4.09 ± 0.30 [c]	1.33 ± 0.20 [c]

[1] Values with the different letters in the column are significantly different ($p < 0.05$) by Tukey's multiple comparison test.

To find a suitable ethanol concentration in the extraction of crocins on GF and GFW, four ethanol concentrations such as 25, 50, 75 and 95% ethanol were investigated synchronously. The yield and total content of crocins obtained by different ethanol concentrations in extraction of GF and GFW are shown in **Table 1**. The results presented that the extraction using 50% ethanol showed significantly potential for extracting crocins than the other ethanol concentrations whether in GW or GFW. The ethanol concentration in the water is beneficial to the increase of the extraction yield which is attributed to moderate polarity of solvent on the nature of crocins., However, too much alcohol concentration is not conducive to the extraction of crocins.

2. The Optimum Ratio of Enzyme to Crocins Required for the Conversion to Crocetin

After the presence of crocins in 50% ethanol extracts being confirmed by HPLC/MS, the next step was to convert the crocins to crocetin by using enzymatic hydrolysis method to increase the yield of TC. **Figure 1** reveals that the ratio 2:1 (crocins solution/enzyme, *v*/*v*) was superior to the other two ratios in releasing TC, liberating more than 80% of theoretical value after 16 h reaction at 50 °C, pH 5.0. The data show that Celluclast 1.5 L contained side activities capable of degrading the water-soluble glycosyl esters, including crocetin di (β-gentiobiosyl) ester. Commercial enzyme cocktails from Novozyme Celluclast 1.5 L have been widely used as sources of endo- and exoglucanases, and β-glucosidase, respectively, for enzymatic hydrolysis [4]. In the study, the researchers have demonstrated that the enzymes Celluclast 1.5 L are applicable for the hydrolysis of crocins to the target of TC.

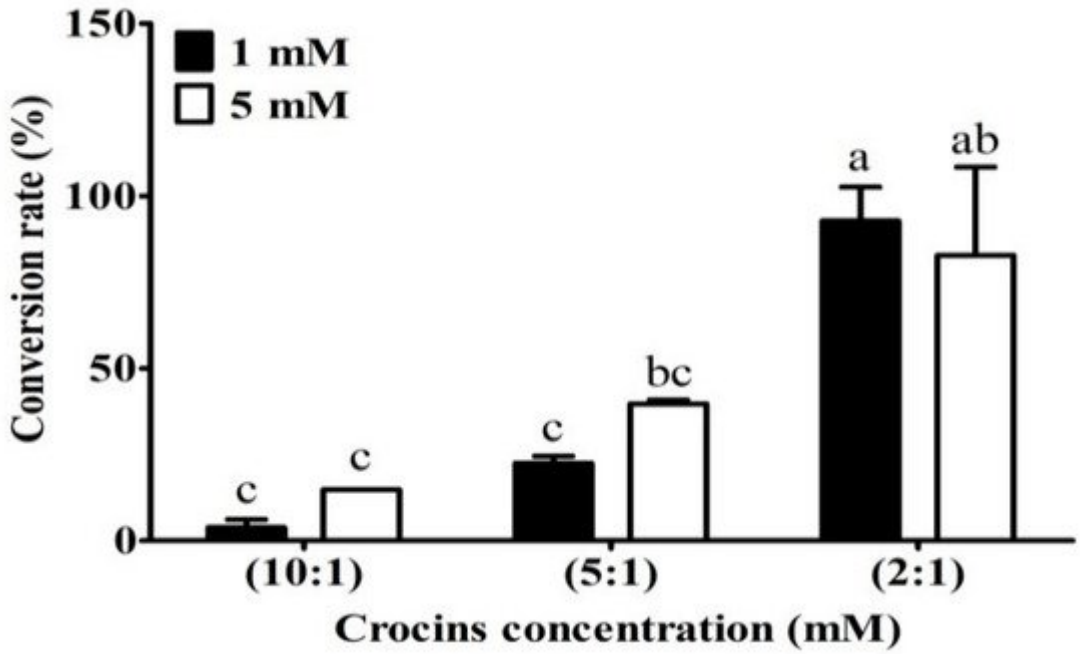

Figure 1. Conversion rate of different concentrations of crocins in enzymes. Crocins (at 1 mM and 5 mM, dissolved in 100 mM of citrate buffer, pH 5.0) and Celluclast® 1.5 L at ratios 10:1, 5:1; and 2:1 (*v*/*v*) were incubated at 50 °C. for 16 h. Different letters in the group indicate significant differences according to the Tukey's multiple comparison test ($p \leq 0.05$).

3. The Optimum Reaction Time Required for the Conversion of Crocins into Crocetin

Celluclast® 1.5 L has been shown a good tolerance to glucose at pH 3.0 and a low Km value [5]. These characteristics were supposed to be capable for application in the deglucosylation of crocins in the study. The reaction system in composed of crocins (10 mg/mL) and Celluclast® 1.5 L at a ratio of 2:1 (*w*/*w*) was incubated at 50 °C for 24 h. The results showed that Cellclast® 1.5 L acted effectively over a 24 h incubation time against the crocins, leading to an approximate of 75% conversion in a rising near to plateau at 16 h (**Figure 2**). **Figure 2** showed the conversion levels with the increasing tendency in a time-dependent manner. In the present study, the two new peaks appeared at 24.92 min (TC) and 25.70 min (*cis*-crocetin) (**Figure 3**, bottom panel) were significantly presented to indicate the hydrolysis activity of the commercial enzymes. However, a limited hydrolysis on *cis*-2-gg (peak at 23.87 min, <15.1%) was encountered in the study. Taken together, the optimum reaction system was found to be the one with a ratio of crocins/Celluclast® 1.5 L = 2:1 (*w*/*w*) and incubated at 50 °C for 16 h (**Figure 2**). The observation indicates the Celluclast® 1.5 L enzymes displayed high stability. In a study of the heat stability of enzymes, the Celluclast 1.5 L suffered only an 18% decrease in its concentration at 50 °C after 4 days, demonstrating remarkable stability at high temperature [4].

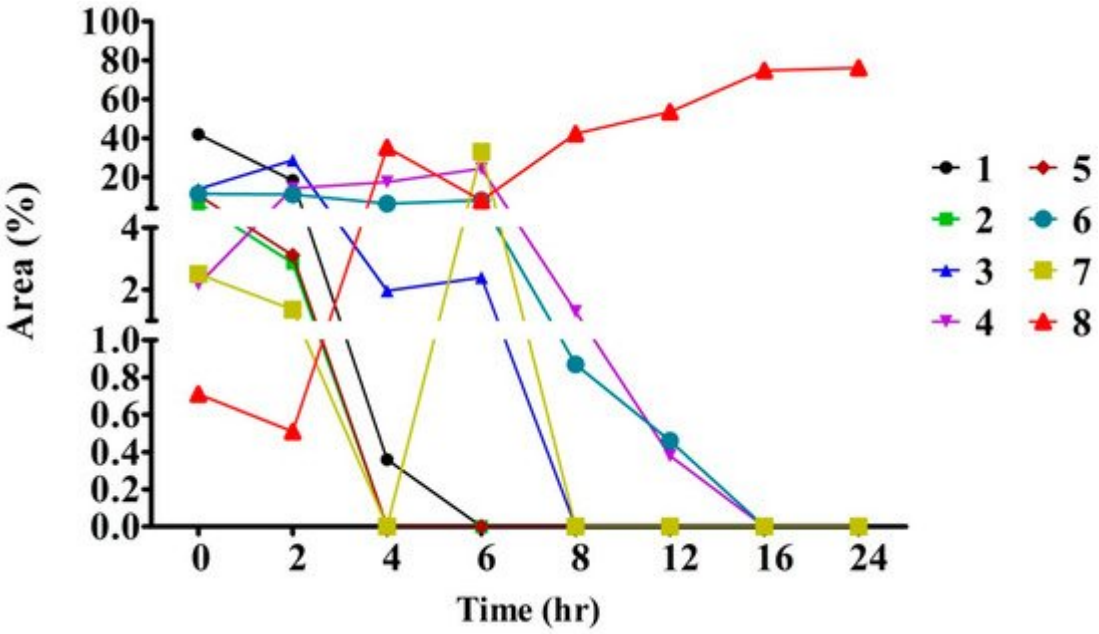

Figure 2. Enzymatic transformation of *trans*-crocetin during a 24 h incubation of crocins extracts from gardenia fruit waste using Celluclast® 1.5 L enzymes. Crocins extracts (2 mM) were reacted with 1.0 mL of Celluclast® 1.5 L enzymes in 10 mL of 100 mM citrate buffer (pH 5.0) at 50 °C. Data presented the averages of 2 experiments as determined by HPLC analysis detected at 440 nm. A description of the numbers is given in **Table 1**.

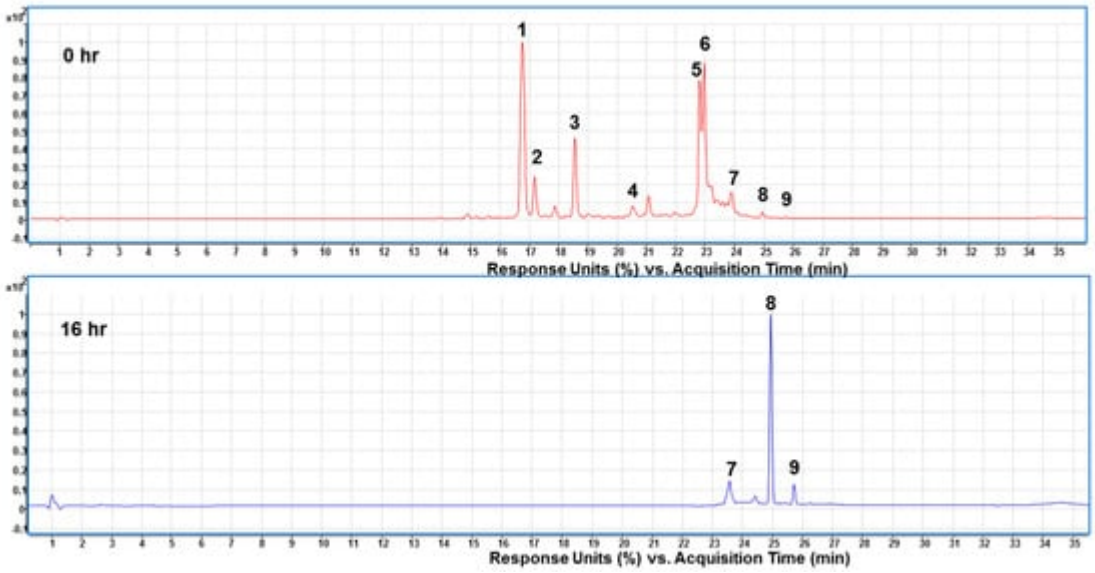

Figure 3. HPLC profiles for the bioconversion of crocins extracts using Celluclast® 1.5 L enzymes detected at 440 nm in the incubation time of 0 and 16 h. HPLC parameters: C18 column (1.8 µm, 2.1 × 100 mm; Agilent Eclipse Plus) column oven 35 °C, mobile phases (**A**) 0.1% formic acid in water and (**B**) acetonitrile (containing 0.1% Formic acid) at 0.3 mL/min. DAD: 440 nm. Peak numbers are referred to **Table 2**.

Table 2. Identification of crocins relative compounds in raw materials and enzyme hydrolyzed products of GFW by using HPLC-DAD-ESI-(+)-MS spectrometry.

Peak No. [1]	Retention Time (min)	λmax (nm)	Molecular Weight	Molecular Ion (*m*/*z*)	Fragmentation (*m*/*z*) [2]	Identified Crocins
1	16.65	438, 466	976.96	999 [M + Na] $^+$	329, 311, 999	*trans*-4-GG [3]
2	17.13	440, 464	976.96	999 [M + Na] $^+$	635, 473, 999	*cis*-4-GG [3]
3	18.51	444, 464	814.82	837 [M + Na] $^+$	327, 837, 311	*trans*-3-Gg [3]
4	20.50	438, 460	652.26	675 [M + Na] $^+$	675, 323, 346	*trans*-2-G [3]
5	22.74	436, 460	976.96	999 [M + Na] $^+$	721, 311, 999	*cis*-4-ng [3]
6	22.91	442, 460	652.26	675 [M + Na] $^+$	675, 311, 329	*cis*-2-G [3]
7	23.87	430, 452	652.26	675 [M + Na] $^+$	675, 228, 329	*cis*-2-gg [3]
8	24.92	426, 450	328.40	329 [M + H] $^+$	329, 311, 293	*trans*-Crocetin [4]
9	25.70	424, 444	328.40	329 [M + H] $^+$	311, 329, 293	*cis*-Crocetin [4]

[1] Peak numbers are referred to **Figure 1** and **Figure 2**. [2] The major fragment ions are ranked in the order of intensity. [3] The tentatively identification of crocins has been reported by Suchareau et al. [6] and Bharate et al. [7]. Namely, G refers to gentiobiose and g, to glucose. [4] The enzyme hydrolyzed products.

References

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