BIOTRANSFORMATION CHARACTERISTICS OF *LORANTHUS TANAKAE* BY *RHODOPSEUDOMONAS PALUSTRIS*

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The orthogonal experiment design and the variation of the transformation time were used to determine the optimal translation conditions and biotransformation characteristics of *Loranthus tanakae* by *Rhodopseudomonas palustris*. High performance liquid chromatography (HPLC) and liquid chromatography – mass spectrometry (LC-MS) were used to analyse the test results. *In vitro* DPPH radical scavenging activity assays were applied to evaluate the antioxidant activities of the converted products. The optimal translation conditions were found to be as follows: pH 8, without conventional medium, and nutrient solution with 10 g/100 mL extract of *L. tanakae* (content of *L. tanakae* extract). Upon the biotransformation of *L. tanakae* by *R. palustris*, the presence of four new chemical components was revealed, indicating that *R. palustris* should have some hydrolytic enzymes to convert four glycosides into four aglucons. *In vitro*, the biotransformation liquid and the contrast liquid offered moderate DDPH radical scavenging activity with IC_{50} values of 45.48 and 67.82 μg/mL, respectively.

Keywords: *Loranthus tanakae*, photosynthetic bacteria (PSB), *Rhodopseudomonas palustris*, biotransformation, orthogonal experimental design

INTRODUCTION

Loranthus tanakae Fr. *et* Sav. (Loranthaceae) is a species of the *Loranthus* semiparasitic plant genus,[1] found in the north and northeast of SiChuan, GanSu, ShanXi, ShanXi, Inner Mongolia, HeBei, ShanDong, at an altitude of 950-2600 m, in mountain broad-leaved forests, growing on the branches of *Quercus* and *Betula* species as host trees.[2,3] The plant contains a variety of natural products from different chemical classes, such as flavonoids, tannin, phenolic compounds, anthraquinones, volatile oil, and sugars.[4] Previous reports[1] have shown that the ethylacetate soluble part of the MeOH extract demonstrated a marginal inhibition on the proliferation of tumor cell lines, such as A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system), and HCT-15 (colon), *in vitro*.

Microbial transformation is an important approach for increasing the structural diversity and biological activity of natural products.[5,6] In previous reports, many transformed products were obtained from natural products,[7,8,9,10,11] indicating that microbial transformation is an efficient approach to achieve structural modification of complicated natural products, which is useful in obtaining more active or less toxic compounds. *Rhodopseudomonas palustris* is a type of photosynthetic bacteria (PSB), which has various enzymes and active metabolites.[12,13,14] There have been a few reports on biotransformation using PSB in the literature,[15,16] but none has been conducted on *L. tanakae*.

The orthogonal experimental design method is a highly efficient way to deal with multifactor experiments and screen optimum levels, which is widely used in the design of statistical experiments in fields as diverse as energy, medicine, materials, and so on.[17,18,19,20,21] In this paper, the orthogonal

experiment design and the variation of the transformation time were adopted to optimize the biotransformation conditions and investigate the biotransformation characteristics of *L. tanakae* by *R. palustris*. In addition, the antioxidant activities of the converted products were studied in the present work.

Table 1
Factors and levels

Level values	Influence factors		
	A (pH)	B (conventional medium concentration)	C (content of *L. tanakae* extract)
1	1 (6.0)	1 (Without)	1 (10 g/100 mL)
2	2 (7.0)	2 (Half)	2 (20 g/100 mL)
3	3 (8.0)	3 (All)	3 (30 g/100 mL)

EXPERIMENTAL

Plant material

Branches of *L. tanakae* were collected from the town of Qinshui, Shanxi Province, China, in October, 2010. The specimen was identified by Prof. Y.E. Bai (School of Pharmacy, Shanxi Medical University, Taiyuan, China) and deposited at the Department of Traditional Chinese Medicine, School of Pharmacy, Shanxi Medical University.

Microorganism

R. palustris was obtained from the College of Life Science and Technology, Shanxi University, China. It was cultivated in a conventional medium for purple non-sulfur bacteria (per liter): 2 g sodium malate, 0.15 g $MgSO_4 \cdot 7H_2O$, 1.2 g yeast extract, and 1.5 g $(NH_4)_2SO_4$; the pH was adjusted to 7.0 before autoclaving.[22] Cultures were incubated at 30 °C with illumination at 2500 Lux under anaerobic conditions. An incandescent light bulb was used. Stock cultures were stored at 4 °C.

Extraction and biotransformation

A sample of dried powdered branches of *L. tanakae* (360 g) was extracted twice by ultrasonic extraction (50% methanol, 1800 mL) at a 30 kHz frequency for 30 min. The extract was evaporated under reduced pressure using a rotary evaporator. The concentrate was diluted with distilled water and used as biotransformation substrate.

A two-stage fermentation protocol[23] was followed for the biotransformation of the *L. tanakae* extract. The microorganisms were transferred from the stock cultures to 500 mL flasks, each containing 450 mL of sterile medium. Preincubation was performed for 48-72 h until suitable growth of the microorganisms was achieved. About 10% of the inoculum derived from the preincubation culture was used to initiate the second-stage culture. The transformation was carried out in 100 mL Erlenmeyer flasks, containing 90 mL of different types of the biotransformation substrate. Cultures were incubated at 30 °C with illumination at 2500 Lux under anaerobic conditions.

Orthogonal design of experiments and the study of biotransformation characteristics

An orthogonal experimental design L_9 (3^4) (shown in Table 1) was used to evaluate the effects of the pH, the conventional medium concentration and the content of *L. tanakae* extract. The culture time was 20 days. The same medium was used for every control group as for each of the orthogonal experiments respectively; it was incubated under the same conditions, but without microorganisms. The results were calculated from the increment of all peak areas of the precipitate, because new chemical components appeared only in the precipitate. The biotransformation characteristics were confirmed by the results of the orthogonal experiment and those of tests 6 and 8, when varying the transformation time (3, 5, 20 days).

Sample preparation

After incubation, all samples were centrifuged into two parts: the supernatant and the precipitate, respectively. Every precipitate was extracted with CH_3OH (3×50 mL) in an ultrasonic extractor for 30 min every time. The extracts were mixed and evaporated under reduced pressure, then the residues were transferred to a 100 mL calibrated flask, diluted to scale with methanol and filtered through 0.45 μm filters prior to the HPLC analysis. The supernatant was disposed with Macroreticular absorbing resin D101, and the methanol eluent was evaporated to dryness under reduced pressure. The residues were transferred to a 100 mL calibrated flask, diluted to scale with methanol and filtered through a 0.45 μm membrane prior to the HPLC analysis.

High Performance Liquid Chromatography analyses

HPLC analyses were carried out on a Shimadzu LC-10ATvp HPLC with UV detector, using a Diamonsil® C_{18}-ODS column (250 mm×4.6 mm, 5 μm, Dikma Technologies). The column was maintained at 25

°C. The detection wavelength was 280 nm. The flow rate was 0.8 mL/min. A gradient elution of A (acetonitrile) and B (water containing 0.2% acetic acid) was used, starting with 5% A and 95% B, to reach 10% A and 90% B at 15 min, 15% A and 85% B at 25 min, 19% A and 81% B at 35 min, 30% A and 70% B at 60 min, 45% A and 55% B at 75 min, 80% A and 20% B at 85 min, and finally 100% A and 0% B at 115 min.

HPLC/ESI-QqTOF MS analyses

Chromatography was performed with an RRLC system (Agilent Technologies, Germany), equipped with a binary solvent manager and a dual pump high pressure gradient setup. The separation was performed in a Diamonsil®C_{18} column (250 mm × 4.6 mm, 5 µm, Dikma Technologies). The detection wavelength was 280 nm at a flow rate of 0.8 mL/min, the column temperature was maintained at 25 °C. The injection volume was 20 µL. The mobile phase A was pure acetonitrile and the mobile phase B consisted of 0.2% acetic acid. The gradient profile was the same as that revealed by the above HPLC analyses. A QTRAP 5500 MS/MS Spectrometer (Applied Biosystem/MDS Sciex, USA) was connected to the RRLC system via an electrospray ionization (ESI) interface. The mass spectrometer was calibrated across the mass range 100–1000 Da, using a solution of sodium formate.

DPPH radical scavenging activity

The samples were incubated under the optimal translation conditions, as revealed by the results of the orthogonal experiment, and the same medium was used for negative controls, which were incubated under the same conditions without any microorganisms for 20 days.

The free radical scavenging activity was analyzed by DPPH photometric assay.[24] 2 mL of test liquid was mixed with 2 mL of 0.5 mM DPPH in ethanol. The absorbance at 517 nm was recorded after 30 min of incubation in the dark at room temperature, using a spectrophotometer. The experiment was performed in triplicates and the percentage antioxidant activity was calculated as follows:

Antioxidant activity [AA] % = 100 - [{(Abs_{sample} - Abs_{blank}) × 100}/$Abs_{control}$]

2 mL ethanol + 2 mL extract was used as blank, while 2 mL 0.5 mM DPPH solution + 2 mL ethanol – as control.

From the inhibition (%), the amount of the samples (µg) reducing the absorbance by 50% was determined (IC_{50}).

RESULTS AND DISCUSSION

In tests 1, 4, 6, 7, 8 and 9 (Table 2), the contents of four components in the control sample of the supernatant decreased obviously after 20 days of culture; these were: quercetin-3-*O*-α-L-rhamnopyranoside (52.30 min), kaempferol-3-*O*-α-L-rhamnopyranoside (58.00 min), rhamnetin-3-*O*-α-L-rhamnopyranoside (70.75 min), rhamnocitrin-3-*O*-α-L-rhamnopyranoside (74.20 min). The structures of these components were elucidated in our previous research and related report.[1] In addition, four new components were observed in the precipitate after 20 days of culture with retention time values of 67.37 min, 75.83 min, 82.41 min and 86.49 min, respectively. By the constituent analysis using LC-MS (Fig. 1) and by comparing the data with those of previous reports,[25] the four new bioconversion products were identified as quercetin (67.37 min), kaempferol (75.83 min), rhamnetin (82.41 min) and rhamnocitrin (86.49 min). In the other test groups, the components underwent minor changes, compared with their respective control groups.

The calculation results of the orthogonal experiment (Table 2) indicated the following range order: $R_A > R_B > R_C$. The order of the factors according to their influence level was as follows: A>B>C, i.e. pH > conventional medium content > content of *L. tanakae* extract. The pH was the uppermost factor and the content of substrate was the factor with the least significance. The selection of the optimum conditions was determined by the increment of all peak areas in the precipitate. From the following results: $k_1<k_2<k_3$ for A, $k_2<k_3<k_1$ for B, and $k_3<k_2<k_1$ for C, we concluded that the optimum conditions were: $A_3B_1C_1$, namely pH 8, without conventional medium, nutrient solution with 10 g/100 mL extract of *L. tanakae*. In addition, the pH ranges over which the enzymes from the bacteria showed activity in this study were similar to the pH ranges of α-L-rhamnosidases from *Aspergillus terreus*[26,27] and *Pseudomonas paucimobilis* FP2001.[28]

Tests 6 and 8 were selected to further investigate the biotransformation characteristics by varying the transformation time (3, 5, 20 days). As the biotransformation time was extended, the changing characteristics of the supernatant and precipitate components could be speculated. In the supernatant of tests 6 and 8 (Figs. 2, 3), the content of the components: quercetin-3-*O*-α-L-rhamnopyranoside (52.30 min), kaempferol-3-*O*-α-L-rhamnopyranoside (58.00 min), rhamnetin-3-*O*-α- L-rhamnopyranoside (70.75 min) and rhamnocitrin-3-*O*-α-L-rhamnopyranoside (74.20 min), was lower than that

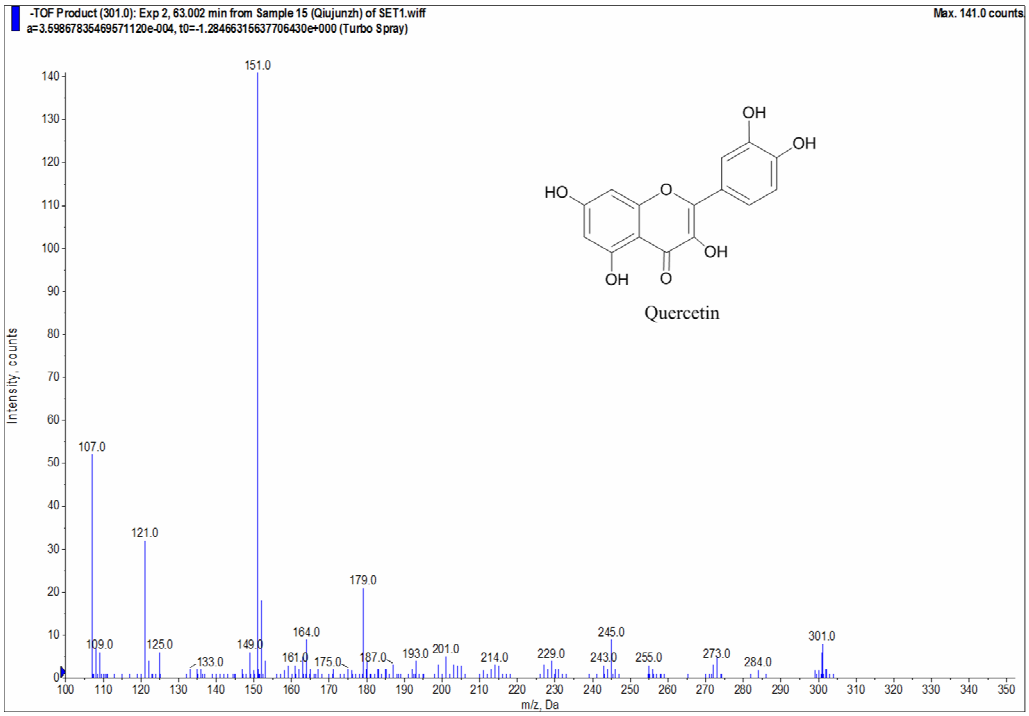
of the control groups on the 3rd day, and obviously decreased over the culture time. Also, in the precipitate of test 6 (Fig. 4), rhamnetin (82.41 min) and rhamnocitrin (86.49 min) were produced on the 3rd day, quercetin (67.37 min) emerged after the 3rd day, and kaempferol (75.83 min) was generated after the 5th day, the amount of growth being remarkable over the culture time. Meanwhile, in the precipitate of test 8 (Fig. 5), quercetin (67.37 min), rhamnetin (82.41 min) and rhamnocitrin (86.49 min) were generated on the 3rd day, and kaempferol (75.83 min) was noted after the 5th day, and although their content increased along with the culture time, the amount of growth was inconspicuous compared with that of test 6. This suggested that the biotransformation in test 8 was basically completed on the 5th day. To sum up, rhamnetin and rhamnocitrin were generated first, then quercetin was produced, and kaempferol was the last component generated. The conditions of test 8 were more beneficial for biotransformation than those of test 6 (Figs. 2-5).

α-L-rhamnosidase cleaves terminal α-L-rhamnose specifically from a large number of natural products. The enzyme has wide occurrence in nature and its presence has been reported in animal tissues, plants, yeasts, fungi and bacteria. It is a biotechnologically important enzyme, due to its applications in debittering and clearance of citrus fruit juices, enhancement of wine aromas and derhamnosylation of many natural products containing terminal α-L-rhamnose to compounds of pharmaceutical interest. Only two commercial preparations of α-L-rhamnosidases (naringinase and hesperidinase) are available, and both are from fungal sources. Most of the reported rhamnosidases were tested on one to four flavonoids of plant origin, naringin, hesperidin, rutin and quercitrin.[28] In naringin, the L-rhamnose is α-1,2-linked to the β-D-glucose, while in hesperidin and rutin it is α-1,6-linked. According to some reported studies, the majority of α-L-rhamnosidases are active on α-1,2 glucosidic linkages, whereas the number of α-L-rhamnosidases active on α-1,6 linkages comes second. There are some α-L-rhamnosidases active on the α-1,4 linkage, but α-L-rhamnosidases active on other glycosidic linkages are rare.[29] Quercitrin has L-rhamnose linked directly to the aglycon. Most of the enzymes are inactive against quercitrin. Only three α-L-rhamnosidases from *Pseudomonas paucimobilis*, *Pichia angusta* X349, and *Fusobacterium* K-60 have been reported to be the most active on the quercitrin of the four substrates.[30,31,32]

Table 2
Orthogonal experiment

Test number	Influence factors			Resulted increment of peak area
	A (pH)	B (conventional medium concentration)	C (content of *L. tanakae* extract)	
1	1 (6.0)	1 (Without)	1 (10 g/100 mL)	67423648
2	1 (6.0)	2 (Half)	2 (20 g/100 mL)	431147
3	1 (6.0)	3 (All)	3 (30 g/100 mL)	-1923446
4	2 (7.0)	1 (Without)	2 (20 g/100 mL)	65668285
5	2 (7.0)	2 (Half)	3 (30 g/100 mL)	68276
6	2 (7.0)	3 (All)	1 (10 g/100 mL)	63942952
7	3 (8.0)	1 (Without)	3 (30 g/100 mL)	119039662
8	3 (8.0)	2 (Half)	1 (10 g/100 mL)	74745434
9	3 (8.0)	3 (All)	2 (20 g/100 mL)	112165497
k_1	7325705.444	28014621.670	22901337.110	
k_2	14408834.780	8360539.667	19807214.330	
k_3	33994510.330	19353889.220	13020499.110	
Range R	26668804.890	19654082	9880838	
Order	A > B > C			
Optimal level	A_3	B_1	C_1	
Optimal combination	$A_3 B_1 C_1$			

k_i is the average targeted value of each experimental factor at the same level i in the orthogonal experiments, which was used to determine the optimal level and the optimal combination of factors; R = max $\{k_i\}$ - min$\{k_i\}$


-TOF Product (301.0): Exp 2, 63.002 min from Sample 15 (Qiujunzh) of SET1.wiff
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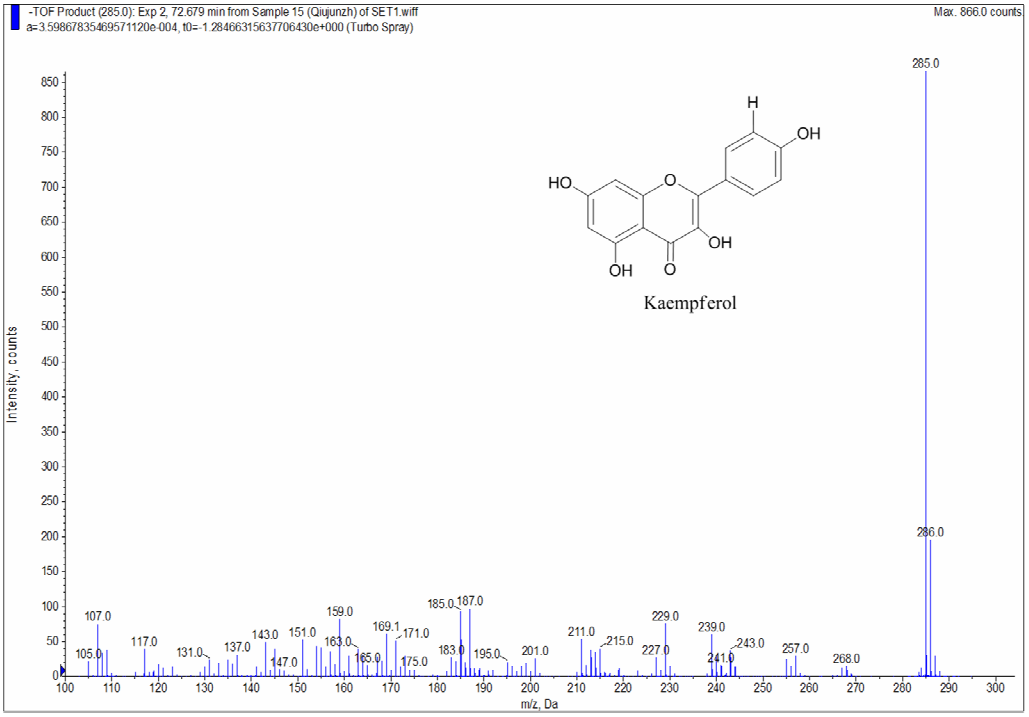
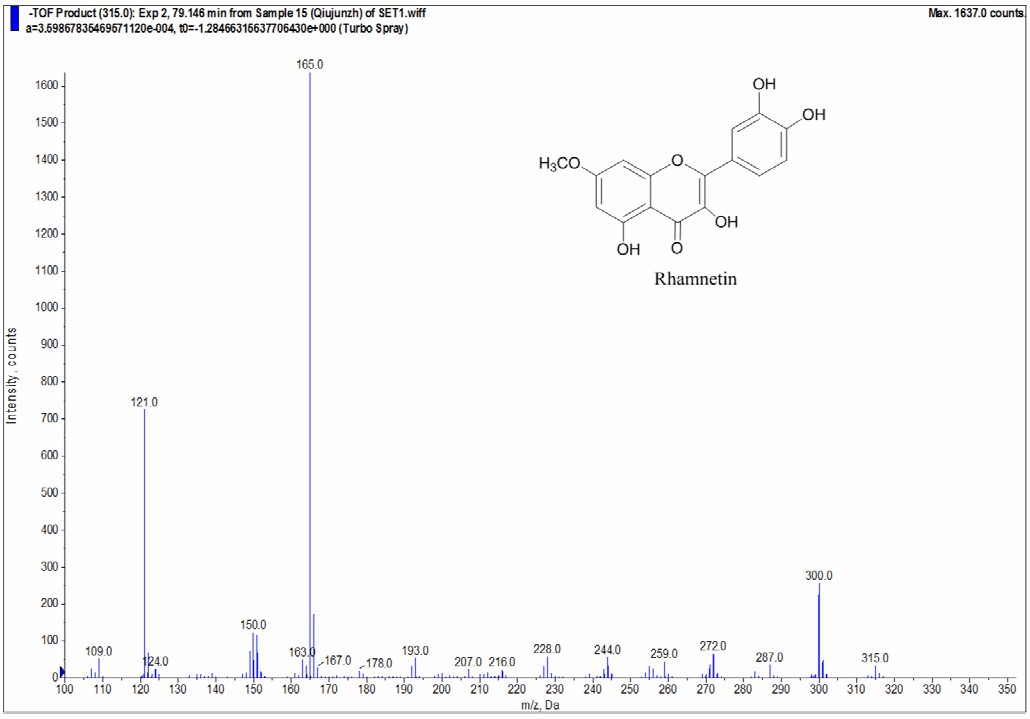
Max. 141.0 counts
Intensity, counts
m/z, Da
151.0
107.0
121.0
179.0
109.0
125.0
133.0
149.0
164.0
161.0
175.0
187.0
193.0
201.0
214.0
229.0
245.0
243.0
255.0
273.0
284.0
301.0
OH
OH
HO
O
OH
OH
O
Quercetin

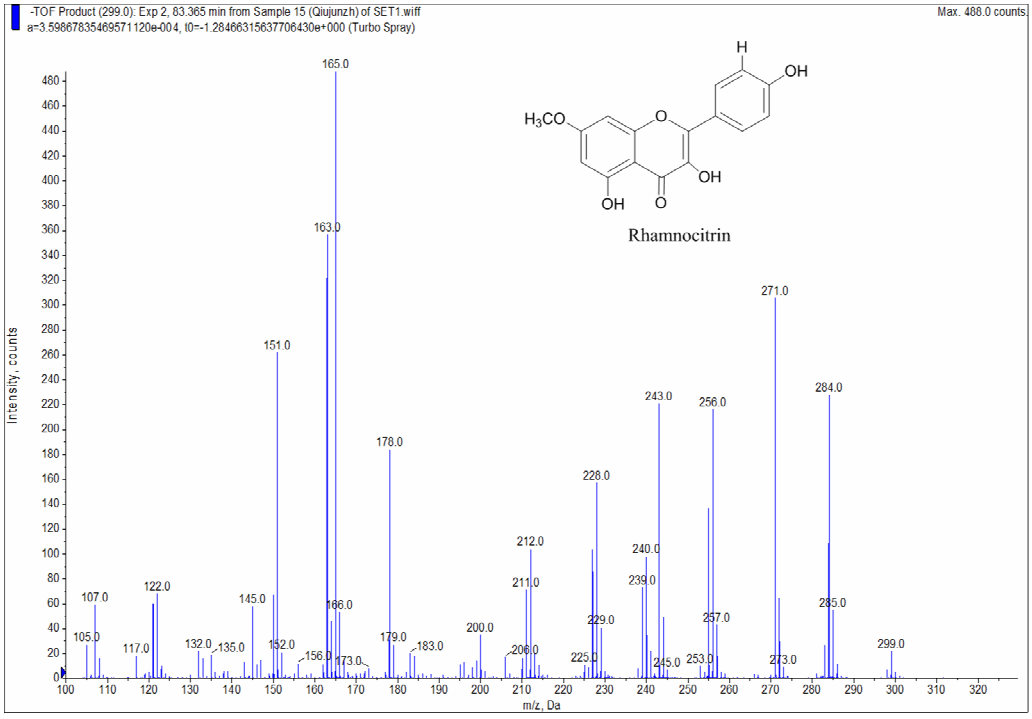
a)


-TOF Product (285.0): Exp 2, 72.679 min from Sample 15 (Qiujunzh) of SET1.wiff
a=3.59867835469571120e-004, t0=-1.28466315637706430e+000 (Turbo Spray)
Max. 866.0 counts
Intensity, counts
m/z, Da
285.0
286.0
107.0
117.0
105.0
131.0
137.0
143.0
147.0
151.0
159.0
163.0
165.0
169.1
171.0
175.0
185.0
187.0
183.0
195.0
201.0
211.0
215.0
227.0
229.0
239.0
241.0
243.0
257.0
268.0
H
OH
HO
O
OH
OH
O
Kaempferol

b)



c)



d)

Figure 1: Mass spectra of new identified constituents: a) quercetin; b) kaempferol; c) rhamnetin; and d) rhamnocitrin

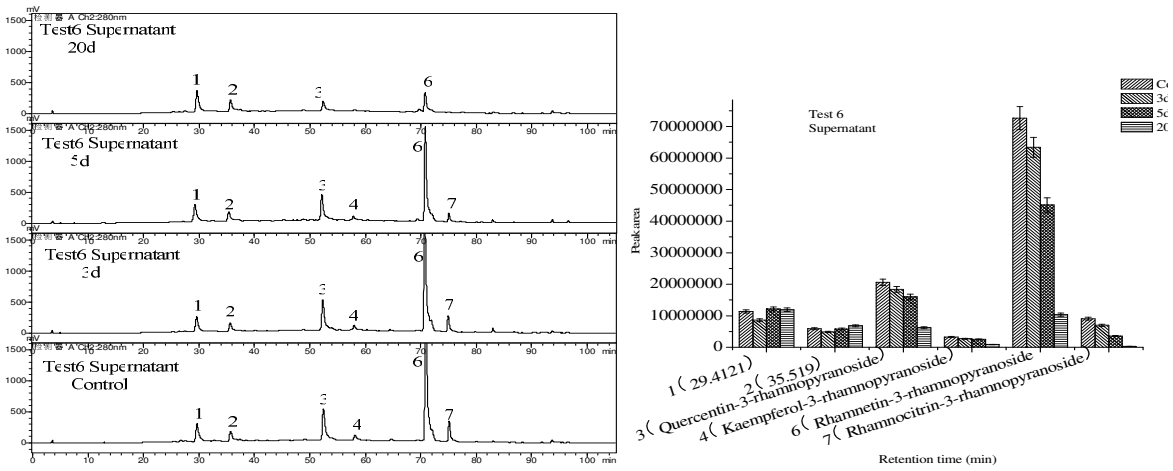


Figure 2: Results for the supernatant of test 6. Left, HPLC chromatograms. Right, the histogram of the major components in HPLC. Compound 3 – quercetin-3-*O*-α-L-rhamnopyranoside, compound 4 – kaempferol-3-*O*-α-L-rhamnopyranoside, compound 6 – rhamnetin-3-*O*-α-L-rhamnopyranoside, compound 7 – rhamnocitrin-3-*O*-α-L-rhamnopyranoside

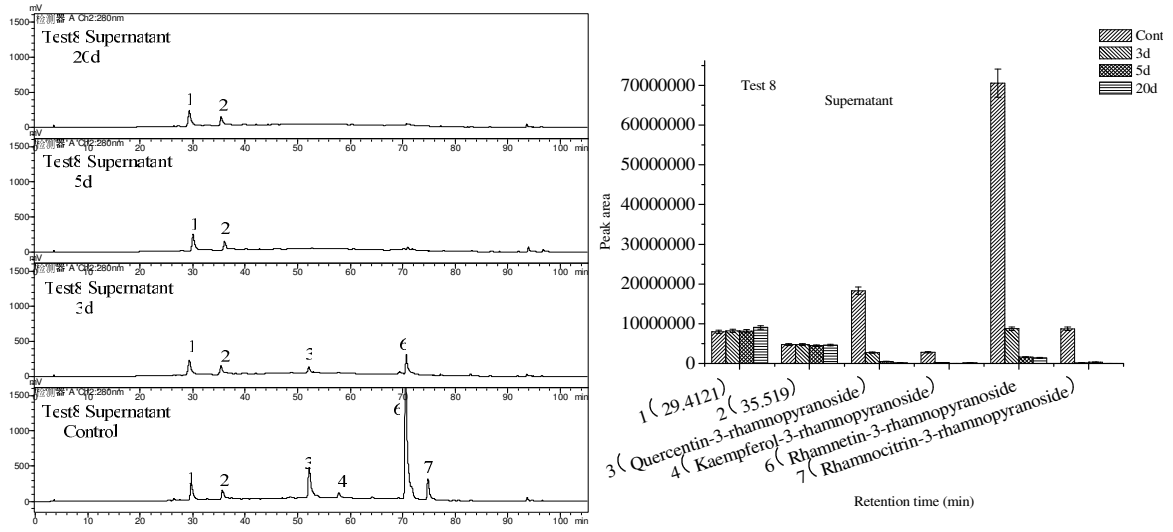


Figure 3: Results for the supernatant of test 8. Left, HPLC chromatograms. Right, the histogram of the major components in HPLC. Compound 3 – quercetin-3-*O*-α-L-rhamnopyranoside, compound 4 – kaempferol-3-*O*-α-L-rhamnopyranoside, compound 6 – rhamnetin-3-*O*-α-L-rhamnopyranoside, compound 7 – rhamnocitrin-3-*O*-α-L-rhamnopyranoside

In our paper, the four glycosides, quercetin-3-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-α-L-rhamnopyranoside, rhamnetin-3-*O*-α-L- rhamnopyranoside and rhamnocitrin-3-*O*-α-L-rhamnopyranoside, which had L-rhamnose linked directly to the aglycon, were hydrolyzed to the corresponding aglycones, but the rutin monomer treated with *R. palustris* under the same conditions was not hydrolyzed. Thus, we inferred that α-L-rhamnosidase from *R. palustris* has a unique substrate in comparison with other known enzymes, which has not been reported before. We performed the BLAST search of *R. palustris*'s genome in NCBI (National Center for Biotechnology Information) and UniGene libraries constructed from our transcriptomics research on

rhamnosidase genes of GH78, but didn't find homologous sequences between them.[33]

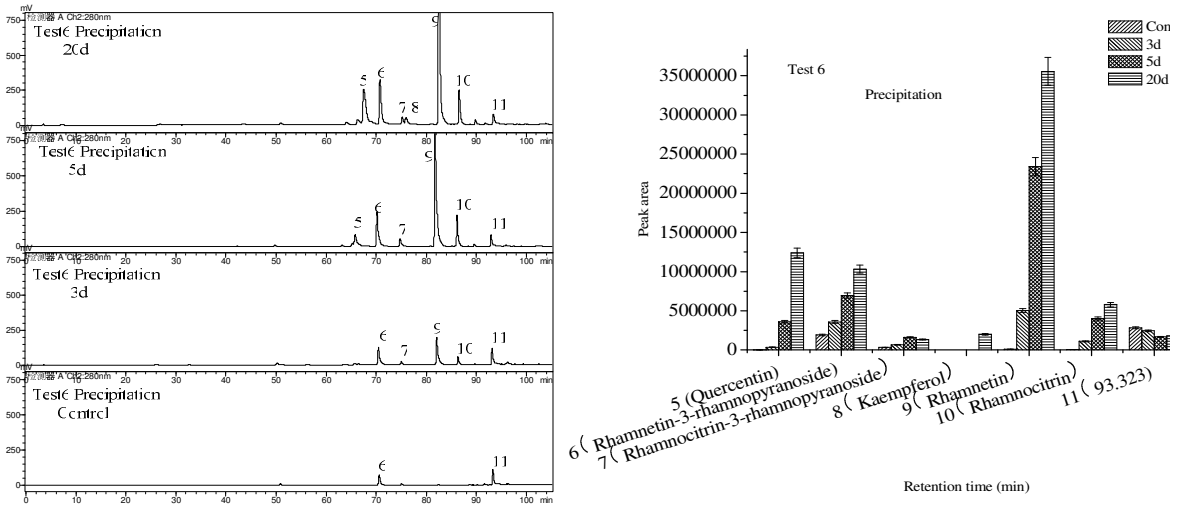


Figure 4: Results for the precipitate of test 6. Left, HPLC chromatograms. Right, the histogram of the major components in HPLC. Compound 5 – quercetin, compound 6 – rhamnetin-3-*O*-α-L-rhamnopyranoside, compound 7 – rhamnocitrin-3-*O*-α-L-rhamnopyranoside, compound 8 – kaempferol, compound 9 – rhamnetin, compound 10 – rhamnocitrin

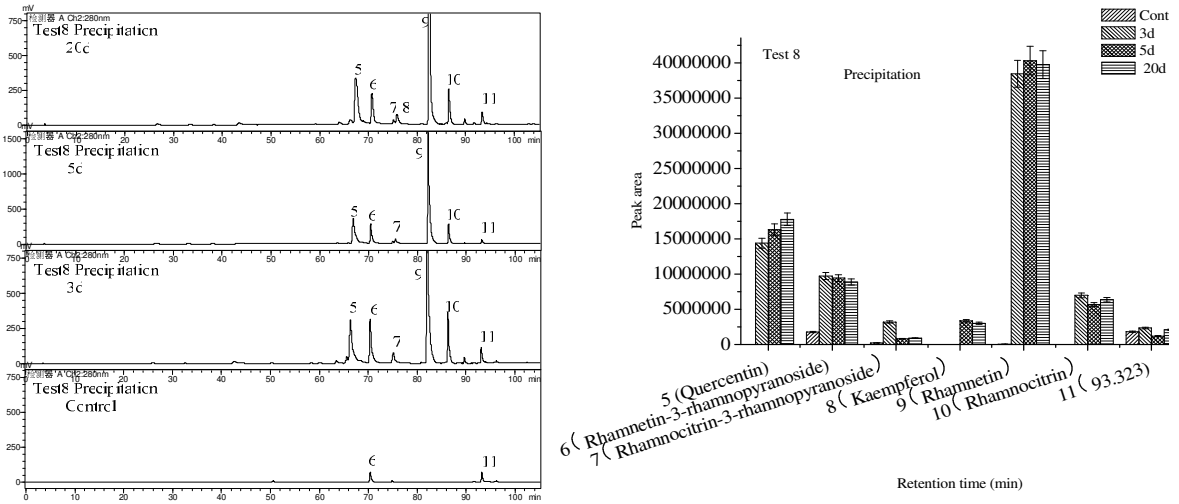


Figure 5: Results for the precipitate of test 6. Left, HPLC chromatograms. Right, the histogram of the major components in HPLC. Compound 5 – quercetin, compound 6 – rhamnetin-3-*O*-α-L-rhamnopyranoside, compound 7 – rhamnocitrin-3-*O*-α-L-rhamnopyranoside, compound 8 – kaempferol, compound 9 – rhamnetin, compound 10 – rhamnocitrin

Thus our further research will focus on metabolomics and enzyme separation to study the new hydrolytic enzymes from *R. palustris*.

It was obvious that the substituent groups of A ring and B ring of the four new compounds were different (Fig. 6). Rhamnetin and rhamnocitrin, which were generated first, contained methoxyl in substituent 7, but rhamnocitrin did not have hydroxy in substituent 3'. Quercetin included hydroxy in substituent 7 and substituent 3', while kaempferol, the last component generated, had hydroxy only in substituent 7. This finding suggests that there should be some relationship between the compound structure and the biotransformation

activity. In the control liquid, the content of the four glycosides was the following in a decreasing order: rhamnetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-α-L-rhamnopyranoside, rhamnocitrin-3-*O*-α-L-rhamnopyranoside and kaempferol-3-*O*-α-L-rhamnopyranoside. It was thus inferred that the rhamnosidase enzyme activity was related to the substituent group, that methoxyl group of A ring was advantageous to the hydrolysis, except to the content. All in all, according to the relationship between the structure and the biotransformation activity order, the following conclusions were drawn: 7-methoxyl and 3'-hydroxy are conducive to biotransformation, while 7-methoxyl is more beneficial to biotransformation than 3'-hydroxy. Further experiments are necessary to confirm this hypothesis because the content of each compound was different in *L. tanakae*, and the concentration and the structural formula of the substrate can greatly affect the conversion rate.[34,35]

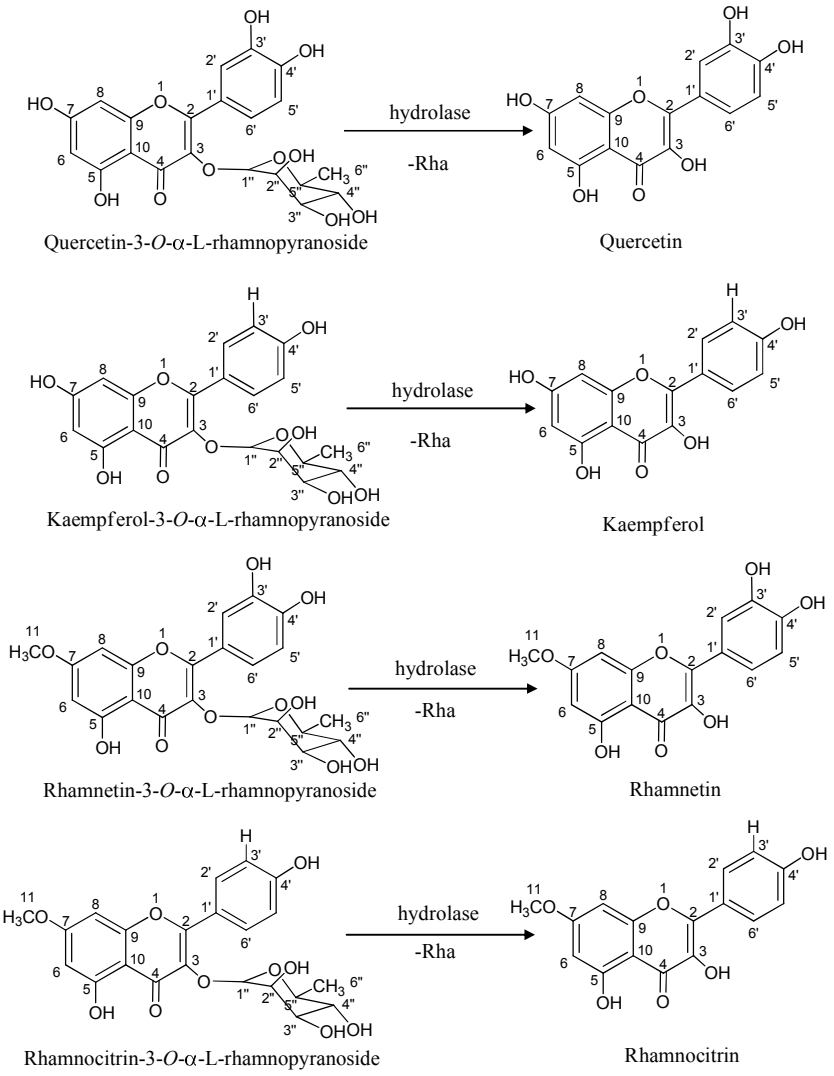


Figure 6: Component structures of *Loranthus tanakae* before and after biotransformation

In our study, the results on the free radical scavenging effects of the biotransformation liquid and the contrast liquid on DPPH indicated moderate activity with IC_{50} values of 45.48 and 67.82 μg/mL, respectively. Through the transformation of flavonoid glycosides, the antioxidant activity of the *L. tanakae* extract was increased, namely the antioxidant activity of flavonoid glycosides could

be enhanced by the hydrolysis of the hydrolytic enzymes, which was consistent with the results reported in the literature.[29,36]

CONCLUSION

The optimal biotransformation conditions of *L. tanakae* by *Rhodopseudomonas palustris* were found to be the following: pH 8, without conventional medium and nutrient solution with 10 g/100 mL extract of *L. tanakae* (the content of *L. tanakae* extract).

Comparing the content changes of the chemical constituents between the supernatant and the precipitate, it was concluded that quercetin-3-*O*-α-L-rhamnopyranoside (52.30 min), kaempferol-3-*O*-α-L-rhamnopyranoside (58.00 min), rhamnetin-3-*O*-α-L-rhamnopyranoside (70.75 min) and rhamnocitrin-3-*O*-α-L-rhamnopyranoside (74.20 min) from the supernatant were used as substrates and converted into quercetin (67.37 min), kaempferol (75.83 min), rhamnetin (82.41 min), rhamnocitrin (86.49 min), respectively, in the precipitate by *R. palustris* (Fig. 6). Thus, *R. palustris* should have one or several kinds of rhamnosidases, which hydrolyzed four glycosides into four aglucons.

In vitro, DPPH was reduced with the addition of biotransformation and the contrast liquid in a concentration-dependent manner within a certain range. When comparing the IC_{50} values ($P < 0.05$), the free radical scavenging potency of the biotransformation liquid was 45.48 μg/mL, which was significantly different from that of the contrast liquid, which was 67.82 μg/mL, indicating that the free radical scavenging effects were enhanced after biotransformation.

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REFERENCES

[1] K. K. Young, S. K. Young, U. C. Sang and Y. R. Shi, *Arch. Pharm. Res.*, **27**, 44 (2004).
[2] X. H. Wang and J. D. Li, *Shandong Sci.*, **14**, 17 (2001).
[3] B. X. Si, R. X. Bai, Y. J. Han, F. X. Zhang and Q. W. Zhang, *Liaoning Forest Sci. Tech.*, **3**, 31 (2008).
[4] N. N. Xu, Y. E. Bai, Q. Q. Xue, S. Y. Zhang and G. E. Yang, *Chin. Remed. Clin.*, **12**, 762 (2012).
[5] G. E. Yang, Z. M. Zhang, H. J. Bai, J. Gong, Y. F. Wang *et al*., *J. Biosci. Bioeng*., **105**, 558 (2008).
[6] Y. Wang, Y. Sun, C. Wang, X. K. Huo, P. D. Liu *et al*., *Phytochemistry*, **96**, 330 (2013).
[7] X. C. Ma, C. K. Sun, S. S. Huang, J. K. Wang, B. J. Zhang *et al*., *Sep. Purif. Technol.*, **76**, 140 (2010).
[8] L. X. Chen, Y. L. Zhuang, L. Shen, E. D. Ma, H. J. Zhu *et al*., *J. Mol. Catal. B-Enzym.*, **72**, 248 (2011).
[9] S. Deng, B. J. Zhang, C. Y. Wang, Y. Tian, J. H. Yao *et al*., *Bioorg. Med. Chem. Lett.*, **22**, 1615 (2012).
[10] X. Lv, D. Liu, J. Hou, P. P. Dong, L. B. Zhuan *et al*., *Food Chem.*, **138**, 2260 (2013).
[11] A. Musa, M. Miao, T. Zhang and B. Jiang, *Food Chem.*, **146**, 23 (2014).
[12] S. Tanskul, O. Kohei, H. S. Oyama, N. Noparatnaraporn, M. H. Tsunemi *et al*., *Biochem. Biophys. Res. Commun.*, **309**, 547 (2003).
[13] F. Badenhop, B. Steiger, M. Sandmann and G. Sandmann, *FEMS Microbiol. Lett.*, **222**, 237 (2003).
[14] H. P. Choi, J. W. Hong, K. H. Rhee and H. C. Sung, *FEMS Microbiol. Lett.*, **236**, 175 (2004).
[15] Y. Ceng and Q. H. Duan, *Biotechnology*, **11**, 44 (2001).
[16] O. I. Aruoma, M. Deiana, A. Rosa, V. Casu, R. Piga *et al*., *Toxicol. Lett.*, **135**, 209 (2002).
[17] M. Dumareya, H. Wikströmb, M. Fransson, A. D. Sparén, P. Tajarobi *et al*., *Int. J. Pharm.*, **416**, 110 (2011).
[18] T. Han, S. X. Cao, L. L. Peng, D. C. Zhu, C. Zhao *et al*., *Opt. Mater.*, **34**, 1618 (2012).
[19] L. Franek and X. Y. Jiang, *Signal Process.*, **93**, 1694 (2013).
[20] P. T. Zhao, S. F. Geand and K. Yoshikawa, *Appl. Energ.*, **112**, 1213 (2013).
[21] L. J. Ji, Y. F. Si, H. F. Liu, X. L. Song, W. Zhu *et al*., *Micropor. Mesopor. Mat.*, **184**, 122 (2014).
[22] Z. Y. Yao and Z. M. Zhang, *Appl. Environ. Microb.*, **2**, 84 (1996).
[23] S. Mathew, T. E. Abramam and S. Sudheesh, *J. Mol. Catal. B-Enzym.*, **44**, 48 (2007).
[24] L. L. Mensor, F. S. Menezes, G. G. Leitão, A. S. Reis, T. C. Santos *et al*., *Phytother. Res.*, **15**, 127 (2001).
[25] Y. Xu, J. Dong, H. Wang, L. R. Wan, Y. S. Rui *et al*., *Chem. J. Chinese U.*, **1**, 46 (2009).
[26] D. Gerstorferová, B. Fliedrová, P. Halada, P. Marhol, V. Krenla *et al*., *Process Biochem.*, **47**, 828 (2012).
[27] L. Weignerová, P. Marhol, D. Gerstorferová and V. Kren, *Bioresour. Technol.*, **115**, 222 (2012).
[28] B. Hákon, O. H. Gudmundur, H. F. Olafur, M. Andrew, K. K. Jakob *et al*., *Enzyme Microb. Tech.*, **34**,

561 (2004).
[29] Y. Vinita, K. Y. Pramod, Y. Sarita and K. D. S. Yadav, *Biochemistry*, **45**, 1226 (2010).
[30] M. Fumio, S. Tomomitsu, F. Y. Hideo Takesue, K. Nobuhiro and W. Kenji, *Arch. Microbiol.*, **173**, 65 (2000).
[31] T. Yanai and M. Sato, *Biosci. Biotechnol. Biochem.*, **64**, 2179 (2000).
[32] S. Park, J. Kim and D. Kim, *J. Microbiol. Biotechnol.*, **15**, 519 (2005).
[33] L. Bi, C. J. Guan, G. E. Yang, F. Yang, H. Y. Yan *et al.*, *Microbiol. Res.*, **185**, 1 (2016).
[34] A. R. Hainal, A. M. Capraru, I. Volf and V. I. Popa, *Cellulose Chem. Technol.*, **46**, 87 (2012).
[35] G. W. Black, T. Gregson, C. B. Mcpake, J. J. Perry and M. Zhang, *Tetrahedron Lett.*, **51**, 1639 (2010).
[36] M. E. Melo Branco de Araújo, Y. E. Moreira Franco, T. Grando Alberto, M. Alves Sobreiro, M. A. Conrado *et al.*, *Food Chem.*, **141**, 266 (2013).