

Comprehensive Profiling of Macamides and Fatty Acid Derivatives in Maca with Different Postharvest Drying Processes Using UPLC-QTOF-MS

Chen Xia,[§] Junlin Deng,[§] Yu Pan,[§] Changbin Lin, Yongqing Zhu, Zhuoya Xiang, Wanyi Li,* Jian Chen,* Yingjiao Zhang, Boyu Zhu, and Qiaolian Huang



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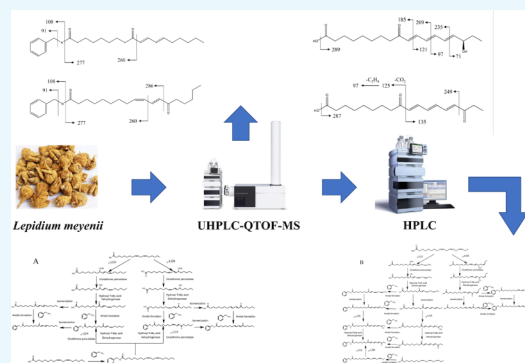
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ABSTRACT: Macamides are characteristically found in maca (*Lepidium meyenii* Walper). Fatty acid derivatives are also an important type of constituent in maca, since they not only relate to the biosynthesis of macamides in the postharvest process but also possess some bioactivities. To study their comprehensive profiles in maca tubers processed via the air-drying method, ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) analyses were performed to identify macamide and fatty acid molecules. Their contents in maca tubers that were processed via air drying and freeze drying, respectively, were further quantified using high-performance liquid chromatography (HPLC) analyses comparing with eight macamide and three fatty acid reference standards. A total of 19 macamides (including four novel ones) and 16 fatty acid derivatives (two found in maca for the first time) were identified. Quantification analyses results showed the eight macamides with contents ranging from 31.39 to 1163.19 $\mu\text{g/g}$ (on dry tuber), and fatty acids from 18.71 to 181.99 $\mu\text{g/g}$ in the air-dried maca, but there were only three macamides and one fatty acid detected with very low contents (3.97–34.36 $\mu\text{g/g}$) in the freeze-dried maca. The results demonstrated that the air-drying method can increase the accumulations of macamides and fatty acids in the metabolism of maca in the postharvest process. The biosynthesis of two types of macamides, i.e., *N*-benzyl-oxo-octadecadienamides and *N*-benzyl-oxo-octadecatrienamides, was further elucidated in detail. These results provide more valuable insights into the phytochemicals of maca, which is helpful to explain its health benefits.



1. INTRODUCTION

Maca (*Lepidium meyenii* Walpers), being native to Peruvian Central Andes, is an important traditional plant in South America. In the past 2000 years, it was also called Peruvian ginseng and used as a functional food for strengthening body and improving sexual performance.¹ Modern studies demonstrated that Maca is a safe food containing numerous nutrients, such as dietary fiber, mineral elements, essential amino acids, vitamins, etc.² Modern phytochemical and pharmacological studies demonstrated that some types of unique constituents in maca, such as macamides (benzylamides of long-chain fatty acids) and macaenes (unsaturated long-chain fatty acid derivatives), are responsible for its various bioactivities.^{2,3} Maca tuber was recommended as a functional food by Food and Agriculture Organization (FAO) in 1992 and has been getting more popular in many countries since then.⁴ In the early 21st century, Maca was successfully transplanted and cultivated in some regions in Yunnan province and Tibet Autonomous Region of China, where the altitude of these regions is similar to that of Peruvian Central Andes. According to the color of its tubers, there are three major types of maca:

black, purple, and yellow. The phytochemicals and health benefits were different between these types of maca, based on their color and cultivation origins.^{5–7}

Because maca is extensively used for functional foods and dietary supplements worldwide, it is significant to comprehensively evaluate the quality, bioactivity, and authentication of maca material, including those cultivated in China. There were several studies focusing on the comprehensive quality evaluation of maca using a variety of analytical technologies, including liquid chromatography-tandem mass spectrometry (LC-MS/MS), inductively coupled plasma-mass spectrometry, and Fourier transform infrared spectroscopy.^{8–11} Determination of macamides, glucosinolates, and fatty acid derivatives

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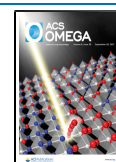


Table 1. Macamides and Fatty Acid Derivatives Identified in the Air-Dried Maca

peak no.	RT (min)	[M - H] ⁻ (m/z)	[M + H] ⁺ (m/z)	formula	mode	fragment ions MS/MS (m/z)	identified compounds	UV max (nm)
1	3.1	307.1909		C ₁₈ H ₂₈ O ₄	-	307, 289, 235, 211, 209, 185, 121	16-hydroxy-9-oxo-10E,12E,14E-octadecatrienoic acid	314
2	4.0	305.1754		C ₁₈ H ₂₆ O ₄	-	305, 287, 249, 135, 125	9,16-dioxo-10E,12E,14E-octadecatrienoic acid	317
3	5.0		398.2703	C ₂₅ H ₃₃ NO ₃	+	398, 380, 273, 260, 108, 91	N-benzyl-16(S)-hydroxy-9-oxo-10E,12E,14E-octadecatrienamide	314
4	6.3		396.2537	C ₂₅ H ₃₃ NO ₃	+	396, 380, 300, 289, 271, 260, 108, 91	N-benzyl-9,16-dioxo-10E,12E,14E-octadecatrienamide	317
5	6.7		234.1863	C ₁₅ H ₂₃ NO	+	234, 108, 91	N-benzyl-octanamide	
6	7.5	293.2114		C ₁₈ H ₃₀ O ₃	-	293, 275, 235, 171, 121	9-hydroxy-10E,12Z,15Z-octadecatrienoic acid	236
7	8.5	291.1956		C ₁₈ H ₂₈ O ₃	-	291, 273, 235, 195, 111, 97	13-oxo-9E,11E,15E-octadecatrienoic acid	276
8	8.9	291.1956		C ₁₈ H ₂₈ O ₃	-	291, 273, 197, 185, 125, 121	9-oxo-10E,12Z,15Z-octadecatrienoic acid	279
9	9.2	291.1963		C ₁₈ H ₂₈ O ₃	-	291, 273, 197, 185, 125, 121	9-oxo-10E,12E,14E-octadecatrienoic acid	276
10	9.4	295.2280		C ₁₈ H ₃₂ O ₃	-	295, 277, 195, 133	13-hydroxy-9Z,11E-octadecadienoic acid	234
11	9.5	295.2271		C ₁₈ H ₃₂ O ₃	-	295, 277, 195, 171, 125	9-hydroxy-10E,12Z-octadecadienoic acid	234
12	10.3	293.2112		C ₁₈ H ₃₀ O ₃	-	293, 275, 249, 195, 185, 113	13-oxo-9Z,11E-octadecadienoic acid	279
13	10.5	293.2117		C ₁₈ H ₃₀ O ₃	-	293, 275, 249, 195, 185, 113	13-oxo-9E,11E-octadecadienoic acid	276
14	10.9	293.2117		C ₁₈ H ₃₀ O ₃	-	293, 275, 249, 197, 185, 125	9-oxo-10E,12Z-octadecadienoic acid	279
15	11.3	293.2117		C ₁₈ H ₃₀ O ₃	-	293, 275, 249, 197, 185, 125	9-oxo-10E,12E-octadecadienoic acid	276
16 ^a	11.6		382.2726	C ₂₅ H ₃₃ NO ₂	+	382, 364, 286, 275, 260, 257, 108, 91	N-benzyl-13-oxo-9E,11E,15E-octadecatrienamide	276
17 ^a	11.9		382.2742	C ₂₅ H ₃₃ NO ₂	+	382, 364, 288, 275, 260, 108, 91	N-benzyl-9-oxo-10E,12Z,15Z-octadecatrienamide	279
18 ^a	12.2		382.2745	C ₂₅ H ₃₃ NO ₂	+	382, 364, 288, 275, 260, 108, 91	N-benzyl-9-oxo-10E,12E,14E-octadecatrienamide	276
19 ^a	13.2		384.2901	C ₂₅ H ₃₇ NO ₂	+	384, 366, 286, 277, 260, 108, 91	N-benzyl-13-oxo-9Z,11E-octadecadienamide	279
20	13.4		384.2896	C ₂₅ H ₃₇ NO ₂	+	384, 366, 286, 277, 260, 108, 91	N-benzyl-13-oxo-9E,11E-octadecadienamideN-benzyl-13-oxo-9E,11E-octadecadienamideN-benzyl-13-oxo-9E,11E-octadecadienamide	276
21	14.0		384.2908	C ₂₅ H ₃₇ NO ₂	+	384, 366, 277, 260, 108, 91	N-benzyl-9-oxo-10E,12Z-octadecadienamide	279
22	14.4		384.2913	C ₂₅ H ₃₇ NO ₂	+	384, 366, 277, 260, 108, 91	N-benzyl-9-oxo-10E,12E-octadecadienamide	276
23	15.9		386.3047	C ₂₅ H ₃₉ NO ₂	+	386, 368, 279, 260, 232, 108, 91	N-benzyl-9-oxo-12E-octadecadienamide	
24	16.4	277.2172		C ₁₈ H ₃₀ O ₂	-	277	(Z,Z,Z)-9,12,15-octadecatrienoic acid	
25	18.5		398.3046		+	398, 138, 121	N-(3-methoxybenzyl)-9Z,12Z,15Z-octadecatrienamide	
26	18.9		368.2949		+	368, 108, 91	N-benzyl-9Z,12Z,15Z-octadecatrienamide	
27	19.0	279.2332		C ₁₈ H ₃₂ O ₂	-	279	(Z,Z)-9,12-octadecatrienoic acid	
28	20.8		400.3208		+	400, 138, 121	N-(3-methoxybenzyl)-9Z,12Z-octadecadienamide	
29	21.1		370.3109		+	370, 108, 91	N-benzyl-9Z,12Z-octadecadienamide	
30	21.2	255.2332		C ₁₆ H ₃₂ O ₂	-	255	1-Pentadecanecarboxylic acid	
31	21.7	281.2494		C ₁₈ H ₃₄ O ₂	-	281	(Z)-9-octadecatrienoic acid	
32	22.6		376.3211		+	376, 138, 121	N-(3-methoxybenzyl)-hexadecanamide	
33	22.9		346.3102		+	346, 108, 91	N-benzyl-hexadecanamide	
34	23.1		402.3367		+	402, 138, 121	N-(3-methoxybenzyl)-9Z-octadecadienamide	
35	23.4		372.3261		+	372, 108, 91	N-benzyl-9Z-octadecadienamide	

^aNovel macamides identified in this study.

using these technologies was efficiently used for the quality assessment and process control of maca.^{12,13} LC-MS/MS could be the best to determine the phytochemical profiles of Maca and authenticate the raw materials combined with multivariate statistical analysis.^{6,10}

Macamides are recognized as a type of characteristic marker for quality evaluation of maca. Maca products contained

abundant macamides that can significantly enhance semen quality and serum hormones, such as luteinizing hormone, estradiol, testosterone, free thyroxin, etc.,¹⁴ suggesting the importance of macamide content for Maca product quality. However, some studies demonstrated that macamide formation is directly related to postharvest processing, including drying process, storage, cleaning, etc.¹⁵⁻¹⁷ Our previous study

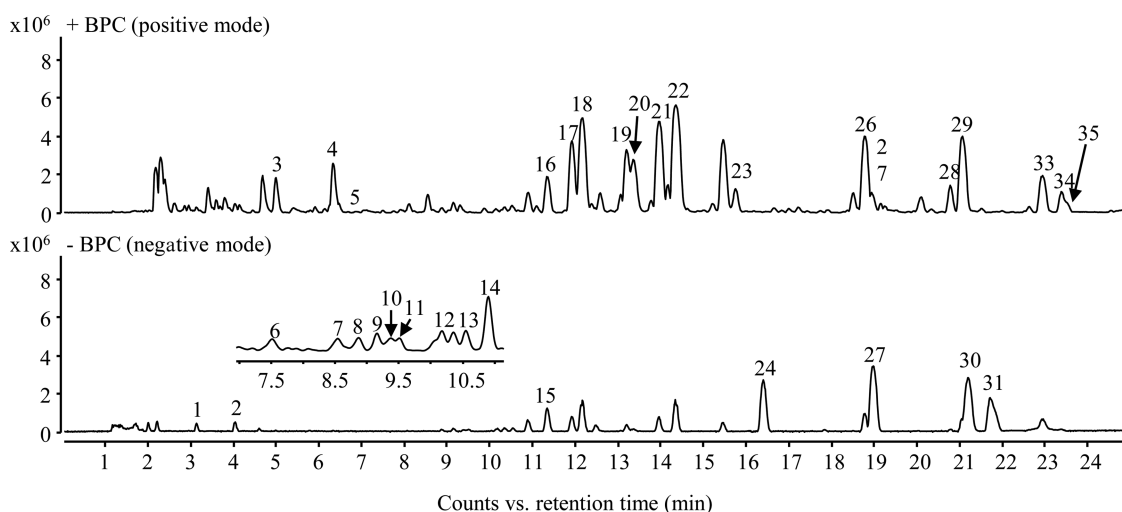


Figure 1. Macamide and fatty acid derivative profiles of air-dried maca.

results also showed that the drying process, especially lyophilization and steaming has a huge impact on the accumulation of macamides and suggested that air drying and oven drying are the appropriate postharvest processing for maca.¹⁸ Chen and his colleagues further optimized the postharvest conditions, indicating that drying temperature, sample forms, and storage period are the key factors for the accumulation of macamides.¹⁵ Given this fact, authentication of maca source based on macamides requires considering the influence of the drying process and storage.

We have preliminarily hypothesized in our previous publication¹² that the formation of macamides in the postharvest drying process is related to macaenes and other long-chain fatty acid derivatives. It was explained that macamides are synthesized via the reaction of benzylamine or its substitutes and long-chain fatty acids catalyzed by enzymes during the drying process. Long-chain fatty acids are the precursors of macamides. Thus, the aims of this study are to clarify the macamide and fatty acid derivative profiles in maca tubers, which were processed via the air-drying method using ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), to quantify their contents in maca tubers, which were processed via air-drying and freeze-drying methods, using high-performance liquid chromatography (HPLC), and to further elucidate the biosynthetic pathways of some macamides, thus to provide more valuable insights of the phytochemicals of maca being helpful to explain its health benefits.

2. MATERIALS AND METHODS

2.1. Chemicals. There were 11 reference standards, including that (Table 1) of compounds 14, 15, 18, 21, and 22, isolated from maca by column chromatography and preparative HPLC in our laboratory. The reference standards of 12, 17, 19, 26, 29, and 33 were synthesized in our laboratory. Their purity was above 98% as tested using HPLC. Their identities were confirmed using the spectroscopic methods nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HR ESI-MS) in a separate publication. Analytical-grade trifluoroacetic acid (TFA) and methanol (MeOH) were bought from Chengdu Kelon Chemical Reagent Factory (Chengdu, China). HPLC-

grade acetonitrile (ACN) was purchased from Sigma (St. Louis, MO).

2.2. Materials and Postharvest Processing. Fresh maca tubers were harvested in a farm at an altitude of 3700 m in Lasa, Tibet Autonomous Region, China, in November 2016, and authenticated by Prof. Wanyi Li. Half of the fresh maca tubers were dried naturally in an open plate at room temperature until they were crisp. The other half of the fresh maca were cut into slices and dried at $-60\text{ }^{\circ}\text{C}$ at a vacuum of 0.1 kPa using an FD-1D-50 freeze dryer (Beijing Bilang Experimental Equipment Co., Ltd., Beijing, China). All of the dried maca tubers and slices were milled into fine powder and stored at $-18\text{ }^{\circ}\text{C}$ for preparation of tests.

2.3. Sample Preparation. Test sample solutions were prepared according to the method described in a previous study,¹⁹ with some modification. Briefly, 1 g of dry maca powder was weighed and homogenized with methanol at a ratio of 1:10 (w/v) and extracted at $40\text{ }^{\circ}\text{C}$ for 1 h with ultrasound assistance. All testing solutions were filtered through $0.22\text{ }\mu\text{m}$ nylon membrane filters before injection into the UPLC-QTOF-MS and HPLC system.

2.4. Identification and Quantification of Macamides and Fatty Acid Derivatives. Identification of UPLC-QTOF-MS analyses was carried out using an Agilent 1290 HPLC system interfaced with Agilent 6545 QTOF MS (Agilent Technologies). The 1290 HPLC system was equipped with a quaternary pump, online vacuum degasser, autosampler, and a diode-array detector. The separation of the compounds was carried out on a Poroshell 120 EC-C18 column ($3 \times 100\text{ mm}^2$, $2.7\text{ }\mu\text{m}$) operated at $40\text{ }^{\circ}\text{C}$. The mobile phase that consists of 0.1% acetic acid (5 mM/L ammonium acetate) in water (A) and acetonitrile (B) was delivered at a flow rate of 0.3 mL/min under a gradient program, which was set at 0–5 min (50–40% B), 5–22 min (40–5% B), and 22–25 min (5% B). The sample injection volume was $2\text{ }\mu\text{L}$. The diode-array detector was set to detect at 280 nm and the online UV spectra were recorded in the scanning range of 200–400 nm.

A mass spectrometer was operated in the negative and positive electrospray ionization mode. Mass spectra were recorded by scanning the mass range of m/z 50–1000 in both MS and MS/MS modes. Nitrogen was used as a drying, nebulizing, and collision gas. The drying gas flow rate was 12 L/min. The heated capillary temperature was set at $350\text{ }^{\circ}\text{C}$ and

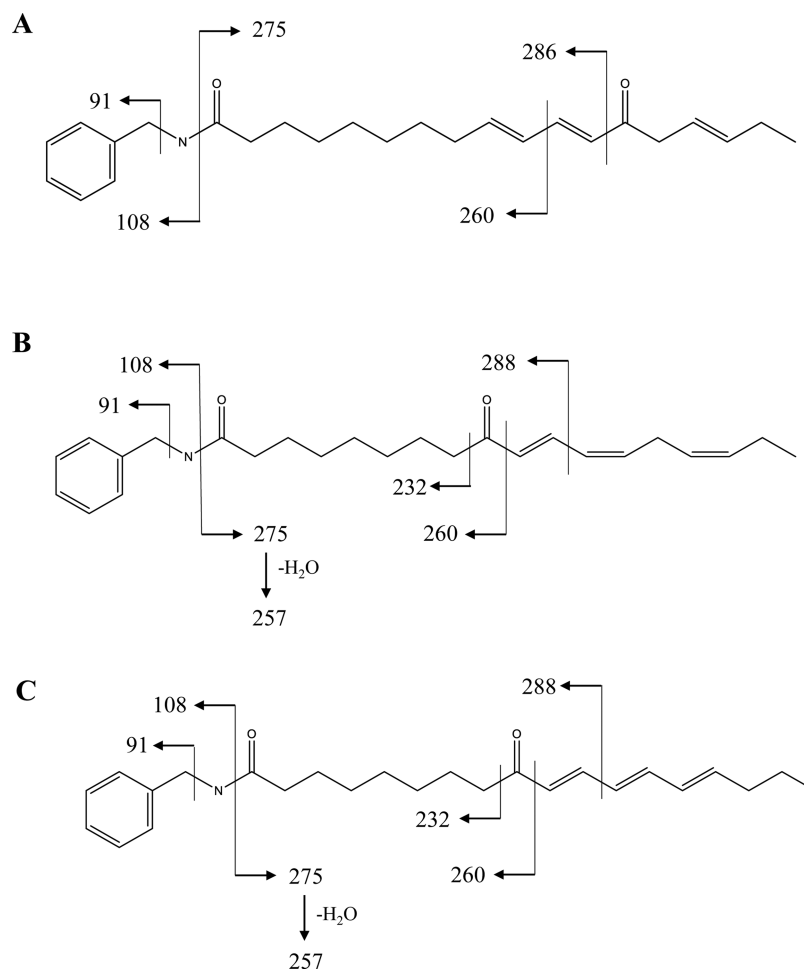


Figure 2. Fragmentation patterns of peak 16 (A), peak 17 (B), and peak 18 (C).

the nebulizer pressure at 45 psi. The source parameters capillary voltage (V_{cap}) and fragmentor, skimmer, and octupole voltages were set to 3500, 175, 65, and 750 V, respectively. For the MS/MS analysis, collision energy was set at 10 and 20 eV. Accurate mass data of the molecular ions were processed through the Mass Hunter version B 04.00 (Agilent Technology Santa Clara, CA).

2.5. Quantification via HPLC. Quantification analyses were performed according to our previous publication with minor revision,¹² using an Agilent 1260 HPLC system (Agilent Technologies). A Zorbax XDB-C18 column (250 mm \times 4.6 mm, 5 μm particle size, Agilent Technologies) was used for separation. The mobile phase consisted of 0.005% trifluoroacetic acid in water (solvent A) and 0.005% trifluoroacetic acid in acetonitrile (solvent B). The gradient program was set at 0–35 min (55–95% B), 35–40 min (95–100% B), and 40–45 min (100% B), with a flow rate of 1 mL/min. The column temperature was 40 $^{\circ}\text{C}$. The injection volume was 10 μL and detection wavelengths were both 210 and 280 nm.

All tests were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Characterization of Macamides and Fatty Acid Derivatives. There are more than 100 compounds that have been identified and characterized in maca and 30% of them are fatty acid derivatives and macamides.^{3,20} A private database of fatty acid derivatives and macamides was established according

to the reported structure. A total of 35 compounds including 16 fatty acid derivatives and 19 macamides in the air-dried maca were tentatively identified by UPLC-QTOF-MS (Table 1). The chemical profiling of air-dried maca was evaluated under optimized chromatographic conditions (Figure 1).

Our previous study indicated that the diagnosed ions of *N*-benzyl-type macamides and *N*-(3-methoxybenzyl)-type macamides were m/z 91 [C_7H_7]⁺ and 108 [$\text{C}_7\text{H}_{10}\text{N}$]⁺ as well as m/z 121 [$\text{C}_8\text{H}_9\text{O}$]⁺ and 138 [$\text{C}_8\text{H}_{12}\text{ON}$]⁺, respectively.¹⁸ This allows us to rapidly distinguish macamides and non-macamides in chemical profiling. The target peaks based on their product ions were divided into three groups: *N*-benzyl-type macamides, *N*-(3-methoxybenzyl)-type macamides, and non-macamides.

For *N*-benzyl-type macamides (peaks 3–5, 16–23, 26, 29, 33, and 35), except for peaks 16–19, most of them were known compounds.^{21,22} Peaks 25, 28, 32, and 34 were the *N*-(3-methoxybenzyl)-type macamides.²³ Characterization was processed using the “Find by formula” algorithm in Agilent Mass Hunter Workstation Software with mass accuracies of <20 ppm between the theoretical mass and calculated mass. The high-resolution mass data, MS/MS fragment ions, and UV maximal absorption wavelengths of identified compounds are listed in Table 1.

According to the UPLC-QTOF-MS data, peaks 16–18 are a group of isomers with the *N*-benzyl nucleus. Their common fragmentation ions are m/z 364, m/z 275, m/z 260, and m/z 257, which are assigned to [$\text{M} - \text{H}_2\text{O} + \text{H}$]⁺, [$\text{M} - \text{C}_7\text{H}_9\text{N} +$

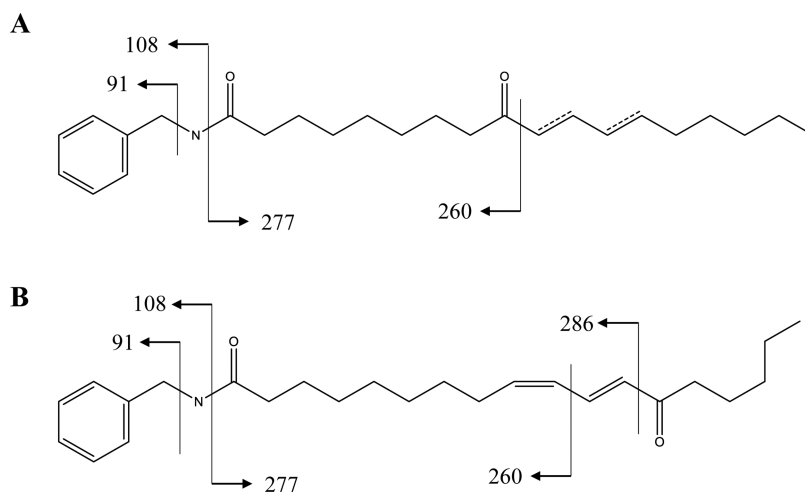


Figure 3. Fragmentation patterns of peaks 21 and 22 (A) and peak 19 (B).

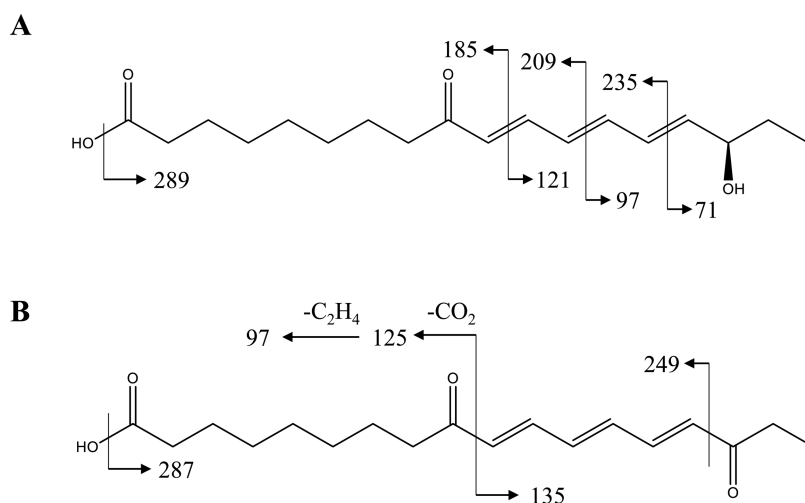


Figure 4. Fragmentation patterns of peak 1 (A) and peak 2 (B).

H^+ , $[\text{M} - \text{C}_9\text{H}_{14} + \text{H}]^+$, and $[\text{M} - \text{C}_7\text{H}_9\text{N} - \text{H}_2\text{O} + \text{H}]^+$, respectively. Peaks 17 and 18 share highly similar fragmentation ions (Table 1). In the same manner, m/z 288 is assigned as $[\text{M} - \text{C}_7\text{H}_{10} + \text{H}]^+$ through losing neutral moieties ($\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{C}\equiv\text{CH}$). The only difference in them is the UV maximal absorption wavelengths, implying peaks 17 and 18 are *cis-trans*-isomers of *N*-benzyl-9-oxo-10,12,15-octadecatrienamides. Their fragmentation patterns are shown in Figure 2. In comparison with the UV maximal absorption wavelengths of peak 14 (λ_{max} 279 nm) and peak 15 (λ_{max} 276 nm), peaks 17 and 18 are identified as *N*-benzyl-9-oxo-10*E*,12*Z*,15*Z*-octadecatrienamide and *N*-benzyl-9-oxo-10*E*,12*E*,14*E*-octadecatrienamide by the reference standards, respectively. For peak 16, m/z 286 was assigned as $[\text{M} - \text{C}_6\text{H}_8\text{O} + \text{H}]^+$, which was derived from cleavage of C12–C13. Compared with the UV maximal absorption wavelengths of peak 18 (λ_{max} 276 nm), peak 16 was tentatively identified as *N*-benzyl-13-oxo-9*E*,11*E*,15*E*-octadecatrienamide.

Additionally, peaks 19–21 were another group of the *N*-benzyl nucleus sharing the same parent ion of m/z 384.2901. Among them, peaks 21 and 22 were identified as *N*-benzyl-9-oxo-10*E*,12*Z*-octadecadienamide and *N*-benzyl-9-oxo-10*E*,12*E*-octadecadienamide based on published data²² (Figure 3A). The characteristic fragmentation ion (m/z 286) $[\text{M} - \text{C}_6\text{H}_8\text{O}$

$+ \text{H}]^+$ was observed in MS² data of peaks 19 and 20 (Figure 3B), but it was absent in that of peaks 21 and 22. By further confirmation using synthetic reference standard, peak 19 was tentatively identified as *N*-benzyl-13-oxo-9*Z*,11*E*-octadecadienamide. Notably, this is the first report of four novel macamides being observed in maca.

Other peaks are fatty acid derivatives displaying the $[\text{M} - \text{H}]^-$ ions in the negative ion mode. In this case, peak 1 (m/z 307.1909) and peak 2 (m/z 305.1754) were selected as examples for the characterization of fatty acid derivatives. According to its fragmentation pattern and literature data,²⁴ peak 1 was tentatively identified as 16-hydroxy-9-oxo-10*E*,12*E*,14*E*-octadecatrienoic acid. A series of cleavages were observed at the conjugated triene structure (C10–C11, C12–C13, and C14–C15), which corresponded to m/z 235 ($[\text{M} - \text{C}_4\text{H}_7\text{O} - \text{H}]^-$), m/z 209 ($[\text{M} - \text{C}_6\text{H}_9\text{O} - \text{H}]^-$), m/z 185 ($[\text{M} - \text{C}_4\text{H}_7\text{O} - \text{H}]^-$), m/z 121 ($[\text{M} - \text{C}_{10}\text{H}_{17}\text{O} - \text{H}]^-$), m/z 97 ($[\text{M} - \text{C}_{12}\text{H}_{17}\text{O}_3 - \text{H}]^-$), and m/z 71 ($[\text{M} - \text{C}_{14}\text{H}_{19}\text{O}_3 - \text{H}]^-$) (Figure 4A). Compared with the fragmentation pattern of peak 1, peak 2 (Figure 4B) was the dehydrogenation product of 16-hydroxy-9-oxo-10*E*,12*E*,14*E*-octadecatrienoic acid, namely, 9,16-dioxo-10*E*,12*E*,14*E*-octadecatrienoic acid.²⁵ Notably, peaks 1 and 2 were also detected in Maca for the first time.

Table 2. Calibrations of the 11 Macamides and Fatty Acid Derivatives

compound	RT (min)	equation	linear range ($\mu\text{g/mL}$)	R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
13-oxo-9Z,11E-octadecadienoic acid	15.52	$Y = 106.6X$	0.25–63.18	0.9998	0.146	0.045
9-oxo-10E,12Z-octadecadienoic acid	16.40	$Y = 107.52X$	0.49–62.5	0.9999	0.145	0.044
9-oxo-10E,12E-octadecadienoic acid	17.09	$Y = 106.99X$	0.28–144.55	0.9999	0.147	0.044
<i>N</i> -benzyl-9-oxo-10E,12Z,15Z-octadecadienamide	17.95	$Y = 82.349X$	0.38–97.80	0.9998	0.159	0.047
<i>N</i> -benzyl-9-oxo-10E,12E,14E-octadecadienamide	18.29	$Y = 81.146X$	0.53–69.24	0.9999	0.164	0.049
<i>N</i> -benzyl-13-oxo-9Z,11E-octadecadienamide	20.54	$Y = 1.301X$	0.34–86.98	0.9998	0.169	0.051
<i>N</i> -benzyl-9-oxo-10E,12Z-octadecadienamide	21.56	$Y = 81.487X$	0.143–73.1	0.9999	0.236	0.071
<i>N</i> -benzyl-9-oxo-10E,12E-octadecadienamide	22.16	$Y = 83.293X$	0.25–127.06	0.9999	0.161	0.048
<i>N</i> -benzyl-9Z,12Z,15Z-octadecatrienamide	29.7	$Y = 56.774X$	0.58–118.5	0.9999	0.275	0.082
<i>N</i> -benzyl-9Z,12Z-octadecadienamide	34.0	$Y = 39.552X$	0.4–118.5	0.9998	0.276	0.083
<i>N</i> -benzyl-hexadecanamide	37.6	$Y = 32.199X$	0.6–220	0.9999	0.272	0.082

Table 3. Contents of Macamides and Fatty Acid Derivatives in Maca Using HPLC Quantification

compounds	content ^a (mean \pm SD $\mu\text{g/g}$)	
	freeze drying	air drying
13-oxo-9Z,11E-octadecadienoic acid	nd	18.71 \pm 0.14
9-oxo-10E,12Z-octadecadienoic acid	3.97 \pm 0.05	77.15 \pm 0.41
9-oxo-10E,12E-octadecadienoic acid	nd	181.99 \pm 0.77
<i>N</i> -benzyl-9-oxo-10E,12Z,15Z-octadecadienamide	nd	58.28 \pm 0.44
<i>N</i> -benzyl-9-oxo-10E,12E,14E-octadecadienamide	nd	199.98 \pm 0.14
<i>N</i> -benzyl-13-oxo-9Z,11E-octadecadienamide	nd	31.39 \pm 0.06
<i>N</i> -benzyl-9-oxo-10E,12Z-octadecadienamide	nd	165.97 \pm 0.73
<i>N</i> -benzyl-9-oxo-10E,12E-octadecadienamide	nd	432.05 \pm 1.45
<i>N</i> -benzyl-9Z,12Z,15Z-octadecatrienamide	34.36 \pm 0.43	354.33 \pm 4.82
<i>N</i> -benzyl-9Z,12Z-octadecadienamide	28.07 \pm 0.75	595.494 \pm 1.04
<i>N</i> -benzyl-hexadecanamide	6.72 \pm 0.38	1163.19 \pm 0.49

^and: not detected.

3.2. Quantification of Macamides and Fatty Acid Derivatives. For comparative studies, 11 macamides and fatty acid derivatives in both air-dried and freeze-dried Maca hypocotyls were examined quantifiably using HPLC. HPLC method calibrations of the 11 compounds are presented in Table 2. The linearity was satisfactory with determination coefficients (R^2) greater than 0.9998 in the concentration range of 0.25–220 $\mu\text{g/mL}$. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on signal-to-noise (S/N) ratios of 3 and 10, respectively.

It is apparent from Table 3 that there were only three macamides and one fatty acid detected with very low contents (3.97–34.36 $\mu\text{g/g}$, on dry tuber, same below) in the freeze-dried maca. On the contrary, the level of macamides and fatty acid derivatives in air-dried maca is far more than that in freeze-dried maca, showing the eight macamides with contents ranging from 31.39 to 1163.19 $\mu\text{g/g}$ and fatty acids from 18.71 to 181.99 $\mu\text{g/g}$. These results are consistent with our previous study.¹⁸ *N*-Benzyl-hexadecanamide (1163.19 \pm 0.49 $\mu\text{g/g}$) exhibited the highest macamide content and 9-oxo-10E,12E-octadecadienoic acid (181.99 \pm 0.77 $\mu\text{g/g}$) was concluded to have the highest contribution to the content of fatty acid derivatives. Although freeze drying could efficiently decrease the drying time, it concurrently interrupted the biosynthetic pathway of macamides. Previous study showed that a negative correlation was observed between postharvest time of maca and glucosinolate content, which is the metabolic precursor of macamides.¹³ The content of macamides was increased with the increase in storage time. In a combination of our results, presumably, the drying process may have a huge impact on the key enzyme catalyzing the formation of macamides. This

suggests that an optimized postharvest promotes the activation of the biosynthetic pathway of macamides.

3.3. Biosynthetic Pathway of *N*-Benzyl-oxo-octadecadienamides and *N*-Benzyl-oxo-octadecatrienamides. According to the quantification analysis, *N*-benzyl-oxo-octadecadienamides and *N*-benzyl-oxo-octadecatrienamides are the most abundant macamides in the air-dried sample. Briefly, macamide formation is based on the amidation reaction of macaenes and benzylamine.¹⁷ In this case, we propose biosynthetic pathways of these macamides as following.

9-Hydroperoxy-10E,12Z-octadecadienoic acid and 13-hydroperoxy-9Z,12E-octadecadienoic acid were synthesized by linoleic acid through catalyzing by lipoxygenase enzymes.²⁶ Then, 9 or 13-hydroperoxy-10E,12Z-octadecadienoic acid was transferred into the basic skeleton of macaenes 9 or 13-hydroxy-10E,12Z-octadecadienoic acid and 9 or 13-oxo-10E,12Z-octadecadienoic acid via catalyzing by glutathione peroxidase and hydroxy fatty acid dehydrogenase. More octadecadienoic acid isomers were generated by isomerization, resulting in the chemical diversity of macamides. In the same manner, linolenic acid was transferred into a variety of octadecatrienoic acid isomers. The putative biosynthetic pathways of *N*-benzyl-oxo-octadecadienamides and *N*-benzyl-oxo-octadecatrienamides are shown in Figure SA,B.

Additionally, the structures of 13-oxo-9Z,11E,15Z-octadecatrienoic acid and *N*-benzyl-13-oxo-9Z,11E,15Z-octadecatrienoamide were unstable and easily converted to 13-oxo-9E,11E,15E-octadecatrienoic acid and *N*-benzyl-13-oxo-9E,11E,15E-octadecatrienamide, respectively, through the *cis*–*trans* isomerization of C12. Because of the reactivity of

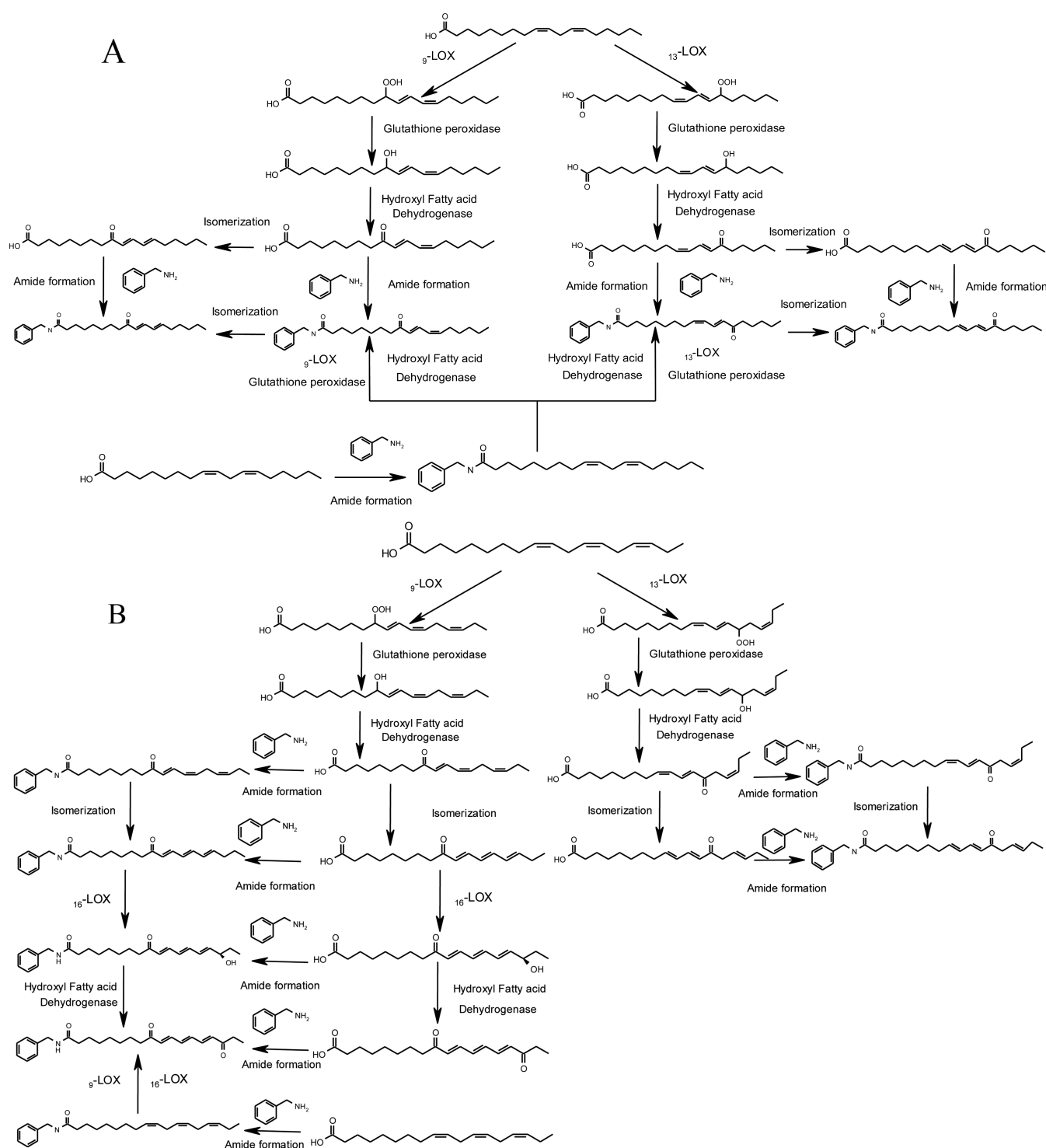


Figure 5. Putative biosynthetic pathways of *N*-benzyl-oxo-octadecadienamides (A) and *N*-benzyl-oxo-octadecatrienamides (B).

allylic hydrogen in 9-oxo-10E,12E,14E-octadecatrienoic acid and *N*-benzyl-9-oxo-10E,12E,14E-octadecadienamides, the hydrogen atom at the C16 position was actively and easily oxidized, leading to the formation of 9-oxo-16-hydroxy-10E,12E,14E-octadecatrienoic acid, 9,16-dioxy-10E,12E,14E-octadecatrienoic acid, *N*-benzyl-16-hydroxy-9-oxo-10E,12E,14E-octadecatrienamides, and *N*-benzyl-9,16-dioxy-10E,12E,14E-octadecatrienamides.

To sum up, the final oxidized products including 9-oxo-10E,12E-octadecadienoic acid, 13-oxo-9E,11E-octadecadienoic

acid, 13-oxo-9E,11E,15E-octadecatrienoic acid, and 9,16-dioxy-10E,12E,14E-octadecatrienoic acid were the most stable derivatives of fatty acids. By the same token, *N*-benzyl-9-oxo-10E,12E-octadecadienamides, *N*-benzyl-13-oxo-9E,11E-octadecadienamides, and *N*-benzyl-9,16-dioxy-10E,12E,14E-octadecatrienamides were the most stable macamides in maca. Although freeze drying has higher dehydration efficiency than air drying, the enzymatic activity related to macamide formation, such as lipoxygenase enzymes, could be reduced due to low temperature and low water content.²⁷ The dramatic decrease of

temperature and water content may inhibit the enzymatic activity and interfere with the reaction of enzymes and substrates, which may provide a rational explanation for low content of macamides in freeze-dried samples. On the contrary, the air-drying process may provide a mild condition for the reaction on macamide formation. Furthermore, other factors, such as pH value, should also be taken into consideration in the future works.

4. CONCLUSIONS

A total of 35 constituents comprised of 19 macamides (including four novel ones) and 16 fatty acid derivatives (two found in maca for the first time) were simultaneously identified in air-dried maca via UPLC-QTOF-MS. Among them, eight macamides (including four novel ones) and three fatty acids were quantified using HPLC-DAD analysis and revealed the differences in constituents between air-dried and freeze-dried maca tubers. Air-dried maca showed higher content levels of macamides and fatty acid derivatives. The possible biosynthetic pathways of *N*-benzyl-oxo-octadecadienamides and *N*-benzyl-oxo-octadecatrienamides in maca tubers during the air-drying process were rationally described. These results provide more valuable insights of the phytochemical metabolism of maca in postharvest processing. The results are also helpful to explain the health benefits of maca.

AUTHOR INFORMATION

Corresponding Authors

Wanyi Li – Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650200 Yunnan, China; Email: 1139382600@qq.com

Jian Chen – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China; orcid.org/0000-0001-8951-1533; Email: 1046652698@qq.com

Authors

Chen Xia – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Junlin Deng – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Yu Pan – Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650200 Yunnan, China

Changbin Lin – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Yongqing Zhu – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Zhuoya Xiang – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Yingjiao Zhang – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Boyu Zhu – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Qiaolian Huang – Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu 610066 Sichuan, China

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsomega.1c02926>

Author Contributions

§C.X., J.D., and Y.P. contributed equally.

Notes

The authors declare no competing financial interest.

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