Process Optimization of Low-temperature Laccase-mediated Lignin Degradation and Dietary Fiber Extraction from Corn Stover

Zheng Kong, Ya Liu and Liying Wang*

Zhuhai College of Science and Technology, Zhuhai Guangdong 519000, China

*Corresponding author's e-mail: lyw07032@zcst.esu.cn
Zheng Kong, Ya Liu and Liying Wang contributed equally to this work

Abstract. Aiming at the low utilization rate of corn stover, high energy consumption and serious pollution of traditional pretreatment methods, and the unclear synergistic mechanism of process parameters in low-temperature laccase-mediated lignin degradation, this study adopted corn stover as raw materials, established the acetyl bromide-ultraviolet spectrophotometry (ABSL) method for lignin quantification, explored the effect of laccase concentration via single-factor experiments, and optimized core process parameters through orthogonal experiments. Results showed that ABSL had excellent linearity (R^2=0.99351) and high precision (RSD<3%). The optimal parameters were 30 °C, 8 h and 60% laccase concentration, with lignin degradation rate of 63.00% and dietary fiber extraction rate of 30.78%. The factor influence order was enzyme concentration > reaction temperature > incubation time. Compared with traditional thermal chemical pretreatment methods, this low-temperature laccase pretreatment method features mild reaction conditions, low energy consumption, high efficiency, low pollution and low cellulose loss, along with environmental friendliness, providing theoretical and technical references for the low-temperature, green and high-value utilization of agricultural straw wastes.

Keywords: Corn stover, Low-temperature laccase, Lignin degradation, Dietary fiber, Acetyl bromide-ultraviolet spectrophotometry (ABSL).

1 Introduction

Global intensification of resource supply–demand imbalance and ecological pressure has rendered efficient green biomass conversion a research hotspot [1]. As abundant agricultural residues in China, corn stover is a high-quality raw material for dietary fiber production owing to its rich cellulose content [2-3]. However, lignin forms a stable cross-linked structure with cellulose and hemicellulose, hindering fiber extraction and restricting the high-value utilization of corn stover [2]. Traditional acid-base and high-temperature pretreatment methods suffer from high energy

© The Author(s) 2026
L. L. Kuan et al. (eds.), *Proceedings of the 2026 3rd International Symposium on Agricultural Engineering and Biology (ISAEB 2026)*, Advances in Biological Sciences Research 56,
https://doi.org/10.2991/978-94-6239-847-4_5

consumption, serious pollution and cellulose loss [4]. Thus, green, mild and efficient pretreatment technologies are urgently required.

Laccase is an environmentally friendly oxidase capable of selective lignin degradation, showing great potential in lignocellulose pretreatment owing to its mild reaction conditions [5-7]. Low-temperature laccase systems consume less energy and cause slight environmental pollution, providing a mild pretreatment method for corn stover [8]. Nevertheless, most current studies focus on single-factor effects on lignin degradation, while the synergistic effects of temperature, time and enzyme concentration remain unclear, limiting the large-scale practical application of this technology.

In this study, an acetyl bromide-ultraviolet spectrophotometry (ABSL) method was established for lignin quantification in corn stover [9]. Single-factor experiments were performed to explore the effect of enzyme concentration on lignin degradation. Orthogonal tests were conducted to optimize key parameters and clarify the synergistic mechanism among parameters. This study provides theoretical and technical references for the green high-value utilization of agricultural residues and broadens the application of low-temperature laccase pretreatment.

2 Materials and Instruments

2.1 Materials and Experimental Reagents

Corn stover (collected from local farmland in Jiaoling County, Meizhou City, Guangdong Province, crushed, dried, sieved, and stored under refrigerated conditions for subsequent experiments); Laccase (*Aspergillus mikutii* source, analytical grade, with an enzyme activity of 10,000 U/g, Hefei Qiansheng Biotechnology Co., Ltd.); Standard lignin (Purity 98%, analytical purity, Hefei BASF Biotechnology Co., Ltd.); PBS (pH 4.5), CH_3COBr, CH_3COOH, NaOH, HCOOH, CH_3CH_2OH. (All the above reagents were of analytical grade and purchased from Shanghai Maclin Biochemical Technology Co., Ltd.).

2.2 Instruments and Equipment

UV2600 spectrophotometer (Shimadzu, Japan); HR/T20M high-speed refrigerated centrifuge (Hunan Huxi Instrument Equipment Co., Ltd., China); FD-1A-50 freeze dryer (Beijing Boyikang Experimental Instrument Co., Ltd., China); ZD-85 constant-temperature shaker (Changzhou Guohua Electric Appliance Co., Ltd., China); DHG-9140A blast drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd., China); PHS-3C pH meter (Shanghai INESA Scientific Instrument Co., Ltd., China).

3 Experimental Methods

3.1 Determination of Lignin Content by ABSL Method

After acetylation, lignin's phenolic hydroxyl groups show a characteristic absorption peak at 280 nm, and their absorbance (A_{280}) is positively correlated with the lignin content, enabling accurate determination of sample lignin concentration via a standard curve [10]. For sample determination: precisely weigh 5 mg of sample powder into a stoppered test tube, add 4 mL of acetyl bromide-acetic acid solution (250 mL/L, v/v), seal the tube, incubate in a constant-temperature water bath for 40 minutes (shaking gently every 10 minutes). After the reaction, cool to room temperature, transfer the mixture to a 50 mL volumetric flask, quench with 12.5 mL glacial acetic acid and 5.0 mL of 0.2 mol/L sodium hydroxide (NaOH), dilute to the mark with glacial acetic acid, measure UV absorbance at 280 nm, and calculate the lignin concentration via the standard curve. To establish the standard curve: prepare standard lignin solutions (0.02, 0.05, 0.08, 0.11, 0.14, 0.17 g/L), be acetylated under the same conditions as the samples, record their A_{280} values, and plot the curve (lignin concentration as abscissa, A_{280} as ordinate) to obtain the linear regression equation and the coefficient of determination (R^2).

3.2 Single- Factor Experiment of Lignin Degradation Mediated by Low-temperature Laccase

To investigate the effect of enzyme concentration on the efficiency of lignin degradation, using the pretreated corn stover powder as the substrate, with a fixed substrate dosage of 2.0 g, 80 mL of PBS buffer (pH 4.5) was added to establish the reaction system. The lignin degradation rate was used as the core evaluation index. The fixed reaction temperature was 24 ℃ and the incubation time was 10 h; the enzyme concentration gradient was set as 0%, 20%, 40%, 60%, 80%, and 100%. Enzyme solutions of desired concentrations were prepared with pure water and transferred together with the substrate into a 50-mL conical flask. The mixture was incubated at a constant temperature in the dark with a shaking speed of 140 rpm. After the reaction, the entire reaction mixture was transferred into a centrifuge tube and centrifuged. The underlying solid residue was collected and dried to constant weight. The obtained samples were acetylated in accordance with Section 3.1. Subsequently, the UV absorbance at 280 nm was determined following the same procedure. The lignin mass fraction was calculated using the standard curve equation, followed by the calculation of the lignin degradation rate.

Calculation Formula:

$$\begin{aligned} \textit{Lignin degradation rate}\,\% = {} & [\textit{Lignin concentration in blank group (0\% laccase concentration)} \\ & - \textit{Concentration in treatment group}] \,/\, \textit{Lignin concentration in blank group} \times 100\% \end{aligned} \tag{1}$$

3.3 Extraction of Dietary Fiber and Calculation of Yield

The saturation inflection point was confirmed through single-factor experiments, and levels around the inflection point were adopted for orthogonal optimization. The temperature and time ranges were formulated based on the study by Rongqian Meng et al. [6], in compliance with experimental data analysis criteria. 2.0 g of pretreated corn stover powder were taken and treated with laccase solutions at different concentrations in accordance with the method described in Section 3.2. The resulting mixture was filtered, dried, and stored for subsequent use. Then, aqueous sodium hydroxide solution was added, the mixture was centrifuged to collect the bottom solid residue, which was washed repeatedly until the pH reached neutrality before centrifugation again. The filter residue was dried and placed in a three-necked flask, followed by the addition of 200 mL of the mixed solution (formic acid: acetic acid: deionized water = 3:5:2, v/v/v). The reaction was conducted at 60 ℃ for 2 hours; after sedimentation, the supernatant was decanted. The precipitate was filtered, and the filter residue was freeze-dried to obtain solid corn stover dietary fiber. The extraction rate was calculated on this basis [9].

Calculation Formula:

$$\text{Dietary Fiber Extraction Rate}(\%) = (\text{Mass of Dietary Fiber After Extraction} / \text{Absolute Dry Mass of Raw Material}) \times 100\% \quad (2)$$

3.4 Orthogonal Experimental Design for Optimization of Degradation Process

Based on the results of single-factor experiments, three key factors, namely reaction temperature (A), incubation time (B), and enzyme concentration (C), were selected, with three levels set for each factor. An orthogonal experimental design was adopted, and the lignin degradation rate was taken as the evaluation index to optimize process parameters for lignin degradation of corn stover by low-temperature laccase and determine the influencing order of each factor. After determining the factor-level combinations, the procedures in 3.1 and 3.2 were repeated to calculate the lignin degradation rate and dietary fiber extraction rate as evaluation indicators. The factor- level table is shown in Table 1.

Table 1. Factors and Levels of Orthogonal Experiment.

Level	Factor A: Reaction Temperature (℃)	Factor B: Incubation Time (h)	Factor C: Enzyme Concentration (%)
1	20	6	60
2	30	8	80
3	40	10	100

3.5 Data Processing

Data sorting and basic calculations were performed using Microsoft Excel and OriginPro 2024. Range analysis of orthogonal experimental data was carried out using Minitab 17 software.

4 Results and Analysis

4.1 Validation Results of Lignin Detection Methods

Verification of the Linear Relationship of the Standard Curve. The linear regression equation of the lignin quantification standard curve established by the ABSL method in this study is: $Y = 40.76571X - 0.01408$, $R = 0.99675$, $R^2 = 0.99351$

As shown in Figure 1, within the concentration range set in the experiment, the lignin concentration and absorbance showed an extremely significant positive linear correlation. The concentration range of the standard curve covered the lignin concentration range of the experimental samples and could meet the requirements for sample quantitative determination.

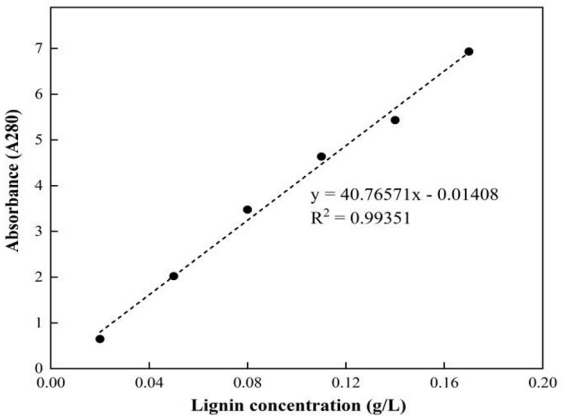


Fig. 1. Quantitative standard curve of lignin by the ABSL method.

Verification of Method Reliability and Precision. Prior to the determination, interfering substances such as HBr and acetic acid produced in the reaction system were neutralized with sodium hydroxide, which eliminated the influence of the reaction medium on the detection results. Meanwhile, sodium bromide has no characteristic absorption near 280 nm and does not interfere with the determination. All samples were determined in three replicate determinations, and the relative standard deviation (RSD) of the parallel samples was less than 3%, indicating good repeatability and stability of the method. In conclusion, the ABSL method has the advantages of simple operation, high accuracy and excellent reliability. It can accurately quantify the lignin content in corn stover samples and provide reliable

scientific data for the evaluation of laccase-mediated lignin degradation efficiency in subsequent experiments.

4.2 Results and Analysis of Single-Factor Experiments

Effects of Enzyme Concentration on Lignin Degradation and Dietary Fiber Extraction. Table 2, Table 3 and Figure 2 present the impacts of varying laccase concentrations on lignin degradation and dietary fiber extraction yield in corn stover. Within the laccase concentration range of 0%–60%, rising enzyme dosage markedly reduced lignin content and significantly increased lignin degradation rate and dietary fiber extraction rate. At 60% concentration, lignin content dropped to 0.0521 g/L with degradation efficiency approaching its maximum value, and dietary fiber extraction rate reached 25.54%. Further concentration increase above 60% yielded no obvious improvement in the two indicators, suggesting a reaction saturation state.

Laccase concentration below 60% could proportionally facilitate lignin degradation, and 60% was confirmed as the saturation concentration of the catalytic reaction. Insufficient active catalytic sites at lower concentrations failed to fully disrupt the lignin structure; excessive enzyme molecules tended to aggregate beyond the optimal concentration, lowering substrate mass transfer efficiency with no extra degradation enhancement and causing waste of enzyme resources. Accordingly, 60% was selected as the optimal enzyme concentration in single-factor experiments.

Table 2. Determination of Standard Lignin by ABSL method.

Group	Enzyme Concentration (%)	UV Absorbance (Abs)	Lignin Concentration (g/L)	Lignin Degradation Rate (%)
M1	0	5.754	0.1415	0.00
M2	20	4.672	0.1150	18.76
M3	40	3.451	0.0850	39.93
M4	60	2.110	0.0521	63.18
M5	80	2.091	0.0516	63.50
M6	100	2.076	0.0513	63.76

Table 3. Dietary fiber yield under different enzyme concentrations.

Group	Enzyme Concentration (%)	Oven-dried raw material mass (g)	Dietary fiber mass (g)	Extraction rate (%)
M1	0	1.542	0.3086	20.01
M2	20	1.483	0.3146	21.21
M3	40	1.536	0.3537	23.03
M4	60	1.498	0.3826	25.54
M5	80	1.513	0.4042	26.72
M6	100	1.526	0.4201	27.53

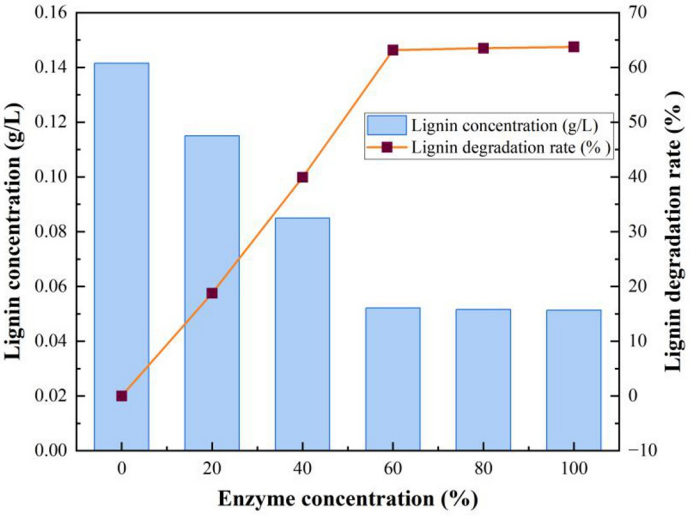


Fig. 2. Analysis of Lignin Degradation Effect under Different Enzyme Concentrations.

4.3 Results and Analysis of Orthogonal Experiment Optimization

Range Analysis of Orthogonal Experiment. The orthogonal experimental results and range analysis with lignin degradation rate selected as the evaluation index are shown in Table 4. A larger range value R means the factor exerts a more significant influence on the lignin degradation rate. The results showed that the influencing order of the three factors on lignin degradation efficiency of corn stover by low-temperature laccase was C > A > B. Enzyme concentration was the dominant factor affecting the degradation efficiency, which was consistent with the single-factor experimental results.

Combined with range analysis and intuitive analysis, the optimal process conditions were determined as A2B2C1, namely a reaction temperature of 30 ℃, an incubation time of 8 h, and laccase concentration of 60%. Under these conditions, the lignin degradation rate reached 63.00%, which was the highest among the nine experimental groups, verifying the optimality of this process combination.

Table 4. Results and Range Analysis of Orthogonal Experiment

Group	Temperature (℃)	Incubation time (h)	Enzyme concentration (%)	UV absorbance (Abs)	Lignin concentration (g/L)	Lignin degradation rate (%)
1	1	1	1	4.421	0.1088	23.11
2	1	2	2	4.113	0.1012	28.45
3	1	3	3	2.790	0.0688	51.39
4	2	1	3	2.443	0.0603	57.40
5	2	2	1	2.120	0.0523	63.00
6	2	3	2	4.335	0.1067	24.60
7	3	1	2	4.472	0.1100	22.23

8	3	2	3	3.746	0.0922	34.81
9	3	3	1	4.540	0.1117	21.05
k1	34.32	34.25	35.72			
k2	48.33	42.09	25.09			
k3	26.03	32.35	47.87			
R	22.30	9.74	22.77			
Degree of influence	2	3	1			

Results of Dietary Fiber Extraction Rate in Each Group of Orthogonal Experiment. The dietary fiber extraction rates of corn stover in nine treatment groups in the orthogonal experiment were determined simultaneously, to clarify the effects of the different process parameters on the dietary fiber yield.

Figure 3 revealed that the dietary fiber extraction rate shared a consistent trend with the lignin degradation rate among all orthogonal experimental groups, indicating a significant positive correlation. The optimal combination A2B2C1 (Test 5) yielded a dietary fiber extraction rate of 30.78%, higher than the other eight groups. As the average of three parallel determinations, this value further verified the superiority of this process for dietary fiber extraction from corn stover.

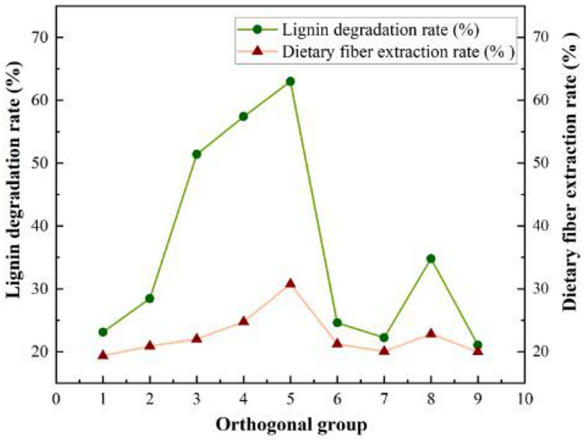


Fig. 3. Variation Rule of Lignin Degradation Rate and Dietary Fiber Extraction Rate under Orthogonal Experimental Conditions.

4.4 Verification Experiment

Three parallel validation trials were performed under optimal conditions: 30 °C reaction temperature, 8 h incubation time and 60% laccase concentration. The average lignin degradation rate was 62.76% and the dietary fiber extraction rate was 30.61%, with the respective RSD values below 2%. The results prove that the low-temperature laccase-mediated lignin degradation process is stable, feasible and highly

reproducible, and can be applied to the efficient extraction of dietary fiber from corn stover.

5 Discussion

This study optimized key parameters via single-factor and orthogonal experiments, clarifying the correlation between laccase-catalyzed lignin depolymerization, structural barrier disruption and improved dietary fiber extraction. Compared with the Klasson method, ABSL is simple, sample-conserving and time-efficient, suitable for high-throughput sample quantification. Consistent with previous biomass research, this method is reliable for determining corn stover lignin content and provides accurate data for subsequent parameter optimization [9-10].

Laccase concentration is the core factor governing lignin degradation efficiency, which reached saturation at 60% enzyme concentration; excessive laccase reduced catalytic efficiency due to steric hindrance and non-specific binding [11]. Orthogonal range analysis confirmed the influence order: enzyme concentration > reaction temperature > incubation time. A temperature of 30 °C preserved laccase activity and 8 h incubation enabled sufficient lignin depolymerization, and the three parameters synergistically achieved optimal degradation and extraction performance.

This study was limited to laboratory scale and has not been scaled up to the pilot scale, and lacked systematic characterization of dietary fiber properties and analysis of lignin degradation by-products. Future research will conduct pilot validation, dietary fiber property characterization and by-product resource utilization to establish a full-component high-value utilization system for corn stover and broaden the application scope of low-temperature laccase pretreatment.

6 Conclusion

A green low-temperature laccase-mediated lignin degradation system was established with corn stover to enhance dietary fiber extraction. Key parameters were optimized via single-factor and orthogonal experiments. The ABSL method allowed for reliable lignin quantification (R^2 = 0.99351, RSD <3%). Laccase concentration was the dominant factor; under optimal conditions (30 °C, 8 h, 60 % laccase concentration), lignin degradation reached 63.00% and dietary fiber extraction rate 30.78%. Low-temperature laccase degraded lignin, broke lignocellulosic barriers, and lignin degradation was positively correlated with fiber extraction. This mild, eco-friendly, low-energy-consumption system provides a green strategy for agricultural residual biomass valorization. Future research will focus on process scale-up and product evaluation for industrialization.

Acknowledgments

This work was funded by the Scientific Research Capacity Improvement Project of Guangdong Key Construction Disciplines (No. 2024ZDJS140). The Laboratory of the School of Life Sciences, Zhuhai University of Science and Technology, provided experimental facilities. The author appreciates Supervisor Liying Wang for her guidance in experimentation and paper writing.

References

1. Guo H, Cao Y, Li H, Xu J, Zhu H. Editorial: Recent advances in biomass conversion to sustainable chemicals and polymers. Front Chem. 2024 Aug 28;12:1475162. doi: 10.3389/fchem.2024.1475162. PMID: 39263588; PMCID: PMC11387986.
2. Qin Y, Fan X, Gao Y, Wang P, Chang J, Liu C, Wang L, Yin Q. Effects of Physicochemical and Biological Treatment on Structure, Functional and Prebiotic Properties of Dietary Fiber from Corn Straw. Foods. 2024 Jun 22;13(13):1976. doi: 10.3390/foods13131976. PMID: 38998482; PMCID: PMC11241159.
3. Kaur G, Kaur P, Devi J, Devi V, Bhushan B, Singh A, Jat HS. Multi-dimensional maize biomass utilization: Mitigating existing agro-waste by meaningful value addition. J Environ Manage. 2025 Aug;390:126232. doi: 10.1016/j.jenvman.2025.126232. Epub 2025 Jun 23. PMID: 40554881.
4. Karnaouri A, Matsakas L, Krikigianni E, Rova U, Christakopoulos P. Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails. Biotechnol Biofuels. 2019 Dec 10;12:285. doi: 10.1186/s13068-019-1628-z. PMID: 31827613; PMCID: PMC6902470.
5. Delugeau L, Grelier S, Peruch F. Enzymatic Treatment of Lignin in Alkaline Homogeneous Systems: A Review on Alkaliphilic Laccases. ChemSusChem. 2025 May 19;18(10):e202402377. doi: 10.1002/cssc.202402377. Epub 2025 Jan 28. PMID: 39815958; PMCID: PMC12094154.
6. Meng R, Zhang S, Zhang Y, Zhao Z, Bao Z, Liu ZH, Zhao ZM, Li BZ. Lignin Fractionation Coupling Laccase-Mediated Depolymerization Enhancing Biological Lignin Valorization. J Agric Food Chem. 2025 Jun 11;73(23):14479-14492. doi: 10.1021/acs.jafc.5c03206. Epub 2025 Jun 1. PMID: 40452236.
7. Haq IU, Saleem A, Chaudhary R, Alessa AH, Nawaz A, Du C. Role of microbial laccases in valorization of lignocellulosic biomass to bioethanol. Front Bioeng Biotechnol. 2024 Jul 23;12:1441075. doi: 10.3389/fbioe.2024.1441075. PMID: 39108598; PMCID: PMC11300222.
8. Song H, Jia H, Wang Q, Zhao X, Yang G, Zhang M, Zhou H, Xu S, Zang Y, Wang Y, Ma L. A New Environmentally-Friendly System for Extracting Cellulose from Corn Straw: The Low Temperature Laccase System. Materials (Basel). 2020 Jan 16;13(2):437. doi: 10.3390/ma13020437. PMID: 31963321; PMCID: PMC7013942.
9. Bozek A, Mathis F, Guillaumat L, Müssig J, Boizot N, Ismael A, Ralph J, Lu F, Sibout R, Beaugrand J. Determination of a suitable molar absorption coefficient (ε) for lignin analysis of fibrous plants using the CASA method. Int J Biol Macromol. 2025 May;308(Pt 1):142324. doi: 10.1016/j.ijbiomac.2025.142324. Epub 2025 Mar 19. PMID: 40118417.

10. Gui J. Acetyl Bromide Method for Total Lignin Content Determination in Plant Biomass. Methods Mol Biol. 2024; 2841:95-100. doi: 10.1007/978-1-0716-4059-3_8. PMID: 39115768.
11. Loi, M.; Glazunova, O.; Fedorova, T.; Logrieco, A.F.; Mulè, G. Fungal Laccases: The Forefront of Enzymes for Sustainability. J. Fungi 2021, 7, 1048. doi: 10.3390/jof7121048.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

