Effect of the Steaming Process on Quality of Postharvest Cistanche deserticola for Medicinal Use during Sun Drying

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Effect of steaming on postharvest stems of Cistanche deserticola Y. C. Ma was evaluated periodically during sun drying in both years. Steamed stems exhibited oily black in color, slightly heavier and longer than untreated ones. The levels of five phenylethanoid glycosides and 2,2-diphenyl-1-picolrylhydrazyl free radical (DPPH) scavenging activity in steamed stems remained relatively stable during sun drying. Steamed samples showed higher amounts of water-soluble extracts, dilute ethanol-soluble extracts, soluble sugars, and polysaccharides, while a lower level of total ashes than untreated samples. The overall results suggest that steaming is an effective processing for enhancing the appearance quality and the concentration of some bioactive compounds in Cistanche Herba.

Key words Cistanche deserticola; steaming; sun drying; postharvest; heat treatment

Cistanche deserticola Y. C. Ma is a holoparasitic plant which often attaches onto the roots of perennial plant Haloxylon ammodendron (C. A. Mey.) Bunge. The dried fleshy stems of C. deserticola is mainly used as Cistanche Herba in China for more than 1800 years. According to Shen Nong Ben Cao Jing, the first Chinese Materia Medica in Eastern Han Dynasty (25–220), Cistanches Herba was documented to treat kidney deficiency, impotence, female sterility, and geriatric constipation. Recently, it is reported that this tonic can advance the ability to learn and memorize, enhance immunity and alleviate fatigue, attracting a large number of consumers in international health food markets. Pharmacological research has demonstrated that phenylethanoid glycosides and saccharides extracted from C. deserticola are the main active components. Phenylethanoid glycosides (acteoside, echinacoside, 2-acetylacecoste, cistanoside A, isoacteoside, etc.) mainly exhibit antioxidative, neuroprotective, and hepatoprotective effects, while polysaccharides and soluble sugars notably show antioxidative, immunological, and anti-aging activity.

The increasing demand has greatly stimulated the cultivation of C. deserticola as a new crop in northwest China. In the majority of the Cistanche-producing regions, postharvest stems are placed directly in open areas for several months for the preliminary drying process. To our knowledge and despite the worry about this extensive farming, no study has been conducted into ST and Control, respectively. Then treatments were randomly collected and divided into two groups for steaming treatment (ST) and untreated control (Control). To conduct ST, stems were held in single layers in a perforated stainless steel steam boiler at 93 ± 1°C for 30 min. Then both steamed and untreated stems were dried on a bamboo mat equipped with a temperature and humidity recorder (HOB® Data Loggers Onset, MA, U.S.A.) in a drying field. At each sampling, 15 stems were randomly collected and divided into 3 groups (n=3). In 2014, 105 stems of C. deserticola were collected. Each stem was cut vertically into 2 equal portions and conducted into ST and Control, respectively. Then treatments were performed the same as that in 2013. In 2013, the first 5 times of samplings continued every 6 d, the last sampling was conducted 12 d later when the moisture of samples descended below 13%. In 2014, the first 6 times of samplings continued every 4 d, the last sampling was conducted 8 d later.

Immediately after sampling, stems were weighed and measured in length and diameter. After coarse-grinding by a JYL-D025 pulverizer (Joyoung, Hangzhou, China), 10 g samples were accurately weighted and used for determining their water content. The remaining samples were stored at −20°C until transported to the laboratory. Coarsely ground bulk C. deserticola samples were homogenized to powder with liquid nitrogen.

Measurement of Morphological Parameters and Water Content At the beginning, every stem was collected to be labeled and measured in weight, length, and diameter (X1). Then at every sampling point, fifteen stems were measured (X1). Loss rates of weight, length, and diameter were cal-

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culated as follows: weight/length/diameter loss (%) = \(\frac{(X_0 - X_1)}{X_0}\) × 100, where \(X_0\) is the initial one and \(X_1\) is the one measured during sun drying. Water content of samples was measured by Method I as described in the Chinese Pharmacopoeia 2015 edition.\(^{18}\)

Measurement of Phenylethanoid Glycosides, Soluble Sugars, Polysaccharides, Dilute Ethanol-Soluble Extracts, Water-Soluble Extracts, and Total Ashes

The methods for measurement of the five phenylethanoid glycosides (acteoside, echinacoside, 2'-acetylaceotside, cistanoside A, and isoacteoside), soluble sugars, polysaccharides, dilute ethanol-soluble extracts were developed and validated in our previous study.\(^{19}\) Measurement of water-soluble extracts was carried out as described in the measurement of dilute ethanol-soluble extracts, using water instead of dilute ethanol. Total ashes were measured by the method as described in the Chinese Pharmacopoeia 2015 edition.\(^{18}\) The concentration of analytes of samples was expressed as in g/kg of dry weight (d.w.). The representative HPLC chromatogram and details of calibration curves are provided in Supplementary materials.

Assay of 2,2-Diphenyl-1-picrylhydrazyl Free Radical (DPPH) Scavenging Activity

The DPPH assay was performed as described in our previous study.\(^{19}\) The results were expressed as micromoles of Trolox per mass of dried stem powder, \(\mu\)mol Trolox Equivalent (TE)/g.

Statistical Analysis

To clarify any differences among sampling dates within each year, one-way ANOVA was applied using SPSS 13.0 (SPSS Inc., Chicago, IL, U.S.A.). The differences were assessed using the Duncan test with a significance limit of 0.05.

RESULTS AND DISCUSSION

Morphological Parameters and Water Content

In two successive years, weight (Fig. 1A) and diameter (Fig. 1B) significantly declined whereas no obvious changes were observed in length (Fig. 1C). Weight decreased by 86% in ST and by 88% in Control. A similar decrease was also seen in the stem diameter by 59% in ST and by 60% in Control. In the experiments reported here, water content declined sharply until approximately 10% was obtained in both years (Fig. 1D). At the last sampling points, dried samples in ST were slightly longer and heavier than the ones in Control. Moreover, ST was significantly more oily black in external color. Since big and oily black colored dried stems are generally believed to be Cistanches Herba with superior quality, we have proved that steaming treatment enhanced external quality of *C. deserticola* slightly.

Phenylethanoid Glycosides and DPPH Scavenging Activity

The levels of echinacoside, cistanoside A, acteoside, isoacteoside, and 2'-acetylaceotside in control and steamed groups at different sampling days are shown in Tables S1 and S2 (Supplementary materials). The changes of total amounts of the five phenylethanoid glycosides are presented in Fig. 2A. In general, phenylethanoid glycoside levels in ST fluctuated during sun drying. However, the concentration of phenyletha-
noid glycosides in Control increased gradually with the water content of stems decreasing. When the water content dropped below 30%, the amounts of phenylethanoid glycosides in Control were higher than the ones in ST. When comparing the concentrations measured at the first sampling point for fresh stems with those at the last sampling point for dried stems, the total amounts of the five phenylethanoid glycosides in Control increased by 88% in 2013 and by 68% in 2014, while no significant changes ($p > 0.05$) in ST were seen in both years. In fact, untreated stems of *C. deserticola* were still live bodies and could perform a variety of biochemical reactions, which was identified by an increasing level of phenylethanoid glycosides during sun drying. Furthermore, phenylethanoid glycosides were reported to be susceptible to temperature variations.20,21) In our study, the trend of air temperature (Fig. S1, Supplementary materials) corresponded well with the changes of total concentration of the five phenylethanoid glycosides in *C. deserticola*. In the experiments reported here, we found that the five phenylethanoid glycosides in untreated stems, especially acetoside and 2′-acetylaceinoside, increased significantly during the sun drying process. As steaming had made most enzymes inactive, phenylethanoid glycosides in ST remained in small fluctuations.

The changes of the DPPH scavenging activity of extracts from *C. deserticola* are noted in Fig. 2B. ST showed a higher capacity to detoxify oxygen radicals than Control in the initial stage, but it reversed in the later period. The trend of the DPPH reaction values was very similar to the change of total levels of the five phenylethanoid glycosides, suggesting that the amount of phenylethanoid glycosides in *C. deserticola* was related to the DPPH scavenging activity.22)

**Soluble Sugars and Polysaccharides** As expected, ST showed higher concentration of soluble sugars (Fig. 3A) and polysaccharides (Fig. 3B) than Control throughout the drying process in both years, with an exception of ones at the first sampling point. Similar to us, Jin *et al.*17) found that steaming led to an increase in reducing sugars and acidic polysaccharides of ginseng, Chang *et al.*16) also found that monosaccharides including galactose and glucose were higher in *Rehmannia glutinosa* roots processed by nine cycles of rice wine immersing, steaming, and drying. Sugars isolated from
Cistanches Herba have been regarded to protect from immunopathological damage and intestinal dysfunction. From our observation, steaming enhanced polysaccharides and soluble sugars in dried stems of *C. deserticola* by 26% and 38%, respectively. The present study suggested that the steaming process on the stems of *C. deserticola* will be useful in the development of Cistanches Herba with the purpose of increasing sugars. During the drying process, an obvious decrease was observed in concentration of soluble sugars in Control, which corresponded well with the increase in the total concentration of five phenylethanoid glycosides. Soluble sugars as primary products of photosynthesis could be biosynthesized into phenylethanoid glycosides, and such conversion disappeared in ST since most enzymes were inactive during the process.

**CONCLUSION**

In conclusion, stems of *C. deserticola* after steaming were oily black in color, slightly heavier and longer. We have demonstrated that the stems processed by steaming showed higher concentration of soluble sugars, polysaccharides, dilute ethanol-soluble extracts, water-soluble extracts, and lower concentration of total ashes. Phenylethanoid glycoside levels and DPPH scavenging activity in steaming treatment remained relatively stable during sun drying, while the ones in untreated control increased gradually. Viewing from marketing and the health points alike, sun drying with steaming is an economical, convenient, and environment-friendly process for *C. deserticola* in producing areas.

There is a significant individual variation in phenylethanoid glycoside levels, thus some researchers cut fresh stems of *C. deserticola* vertically into equal portions for different treatment groups. In the present study, similar changes in the quality of *C. deserticola* were obtained from the whole stems in 2013 and the half portions in 2014. Material form did not significantly affect the concentration of phenylethanoid glycosides, soluble sugars, polysaccharides, dilute ethanol-soluble extracts, and water-soluble extracts in fresh or steamed stems, so for their characterization, no particular precautions are required.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**REFERENCES**

1. Wang T, Zhang XY, Xie WY. *Cistanche deserticola* Y. C. Ma, "Desert ginseng": a review. *Am. J. Chin. Med.*, **40**, 1123–1141 (2012).
2. Jiang Y, Tu PF. Analysis of chemical constituents in *Cistanche* species. *J. Chromatogr. A*, **1216**, 1970–1979 (2009).
3. Wang LL, Ding H, Yu HS, Han LF, Lai QH, Zhang LJ, Song XB. *Cistanches Herba*: chemical constituents and pharmacological effects. *Chinese Herbal Medicines*, **7**, 135–142 (2015).
4. Xiong Q, Kadota S, Tani T, Namba T. Antioxidative effects of phenylethanoids from *Cistanche deserticola*. *Biol. Pharm. Bull.*, **19**, 1580–1585 (1996).
5. Luo L, Wu XC, Gao HJ, Lv SZ, Wang JH, Wang XW. Protective effect of total glycosides of *Cistanche* Herba on Alzheimer's disease rats. *Chin. Pharm.*, **24**, 2122–2125 (2013).
6. Luo L, Tuexun A, Wang XW. Protective effects of glycosides of *Cistanche* on apoptosis of PC12 cells induced by aggregated β-amyloid protein 25–35. *Chin. J. New Drugs Clin. Res.*, **29**, 115–118 (2010).
7. Xiong Q, Hase K, Tezuka Y, Tani T, Namba T, Kadota S. Hepato-protective activity of phenylethanoids from *Cistanche deserticola*. *Planta Med.*, **64**, 120–125 (1998).
8. Sui ZF, Gu TM, Liu B, Peng SW, Zhao ZL, Li L, Shi DF, Yang RY. Water-soluble carbohydrate compound from the bodies of *Herba Cistanches*: isolation and its scavenging effect on free radical in skin. *Carbohydr. Polym.*, **85**, 75–79 (2011).
9. Wang XY, Qi Y, Cai RL, Li XH, Yang MH, Shi Y. Enhancing effect of polysaccharides on *Cistanche deserticola* Y. C. Ma on lymphocyte proliferation. *Acta Lab. Anim. Sci. Sin.*, **17**, 424–427 (2009).
10. Dong Q, Yao J, Fang JN, Ding K. Structural characterization and immunological activity of two cold-water extractable polysaccharides from *Cistanche deserticola* Y. C. Ma. *Carbohydr. Res.*, **342**, 1343–1349 (2007).
11. Wang XY, Qi Y, Cai RL, Li XH, Yang MH, Shi Y. The effect of *Cistanche deserticola* polysaccharides (CDPS) on macrophages activation. *Chin. Pharmacol. Bull.*, **25**, 787–790 (2009).
12. Gao JY, Jiang Y, Dai F, Han ZL, Liu HY, Bao Z, Zhang TM, Tu PF. Study on laxative constituents in *Cistanche deserticola* Y. C. Ma. *Mod. Chin. Med.*, **17**, 307–310, 314 (2015).
13. Xue DJ, Zhang M, Wu XH, Chen XD, Zhan YC. Studies on the active antisenile constituents in *Cistanche deserticola* Y. C. Ma. *Chin. J. Chin. Mater. Med.*, **20**, 687–689, 704 (1995).
14. Xu R, Chen J, Chen SL, Liu TN, Zhu WC, Xu J. *Cistanche deserticola* Ma cultivated as a new crop in China. *Genet. Resour. Crop Evol.*, **56**, 137–142 (2009).
15. Liu ZL, Cao ZM, Liu YY, Song ZQ, Lu AP. Maillard reaction involved in the steaming process of the root of *Polygonum multiflorum*. *Planta Med.*, **75**, 84–88 (2009).
16. Chang WI, Choi YH, Van Der Heijden R, Lee MS, Lin MK, Kong HW, Kim HK, Verpoorte R, Hankemeier T, Van Der Greef J, Wang M. Traditional processing strongly affects metabolite composition by hydrolysis in *Rehmannia glutinosa* roots. *Chem. Pharm. Bull.*, **59**, 546–552 (2011).
17. Ju Y, Kim YJ, Jeon JN, Wang C, Min JW, Noh HY, Yang DC. Effect of white, red and black ginseng on physicochemical properties and ginsenosides. *Plant Foods Hum. Nutr.*, **70**, 141–145 (2015).
18. Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People's Republic of China 2015*. Volume IV, China Medical Science Press, Beijing, pp. 104, 202, 204 (2015).
19. Peng F, Chen J, Wang X, Xu CQ, Liu TN, Xu R. Changes in levels of phenylethanol glycosides, antioxidant activity, and other quality traits in *Cistanche deserticola* slices by steam processing. *Chem. Pharm. Bull.*, **64**, 1024–1030 (2016).
20. Wu YT, Lin LC, Sung JS, Tsai TH. Determination of acteoside in *Cistanche deserticola* and *Boschniakia rossica* and its pharmacokinetics in freely-moving rats using LC-MS/MS. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **844**, 89–95 (2006).
21. Jia CQ, Shi HM, Wu XM, Li YZ, Chen JJ, Tu PF. Determination of echinacoside in rat serum by reversed-phase high-performance liquid chromatography with ultraviolet detection and its application to pharmacokinetics and bioavailability. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **844**, 308–313 (2006).
22. Cheng XY, Wei T, Guo B, Ni W, Liu CZ. *Cistanche deserticola* cell suspension cultures: Phenylethanol glycosides biosynthesis and antioxidant activity. *Process Biochem.*, **40**, 3119–3124 (2005).
23. Du Y, Guo YH, Cui XS, Chen XL, Zhai ZX. Technology of air-impingement drying for fresh *Cistanche*. *T. Chin. Soc. Agric. Eng.*, **26**, 334–337 (2010).