

Chemical differences between steamed rhubarbs with or without pre-processing with liquor

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Abstract

In China, rhubarb is processed by several methods to enhance particular desirable effects and reduce side effects. One of the resultant products is known as ‘*Jyuku-daiou*’ (JD) in Japanese (*Shu-dahuang* in Chinese). In ancient times, there were two methods for preparing JD, steaming and steaming after processing with liquor (liquor-steaming). At present, JD refers to liquor-steamed rhubarb; therefore, the medicinal effect of JD may have changed. In this report, we elucidated the medicinal effects of steamed (St) rhubarb and liquor-steamed rhubarb by searching the Chinese medical literature from ancient times. Next, we compared the contents of the principal compounds in St rhubarb and two types of liquor-steamed rhubarb, rhubarb sprayed with 16% ethanol (as a substitute for *huangjiu*) before being steamed (LSp + St) and rhubarb soaked in 16% ethanol before being steamed (LSO + St).

In herbological study, we found that JD was used as a remedy for poison, improving blood stasis, and moistening the bowel in ancient times. Furthermore, in chemical study, we found that St and LSp + St had decreased sennoside contents, while LSO + St had an increased anthraquinone content and a decreased sennoside content. Therefore, LSO + St is the only process that increases the contents of anthraquinones, which have anti-bacterial and anti-inflammatory effects that have been linked to detoxification effect, and we think that all processed rhubarbs may have relatively strong blood stasis improving effects due to their decreased sennoside contents. However, we could not confirm that processed rhubarb moistens the bowel.

Key words rhubarb, processing, steaming, sennoside, anthraquinone, medical literature.

Abbreviations JD, *jyuku-daiou*; LSO + St, rhubarb soaked in 16% ethanol before being steamed; LSp + St, rhubarb sprayed with 16% ethanol before being steamed; St, steaming.

Introduction

Rhubarb is a crude drug with many medicinal effects, e.g., purgative effects^{1,2)} due to sennoside, anti-bacterial³⁾ and anti-inflammatory effects⁴⁾ due to anthraquinone, anti-inflammatory effects⁵⁾ due to lindleyin, and radical scavenging effects⁶⁾ due to tannin. In China, rhubarb is processed to enhance particular desirable effects and reduce side effects. In ancient times,

rhubarb dipped in liquor (liquor-dipped rhubarb) and rhubarb soaked in liquor (liquor-soaked rhubarb) were used.^{7,8)} In modern times, rhubarb sprayed with liquor before being fried, which is known as ‘liquor-rhubarb’; rhubarb sprayed with liquor before being steamed, which is known as ‘*Jyuku-daiou* (JD) in Japanese (*Shu-dahuang* in Chinese)’; and rhubarb burned until it has been reduced to charcoal, which is known as named ‘rhubarb-coal’, are documented in the Chinese Pharmacopoeia.⁹⁾

We previously found that liquor-soaking was

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replaced by liquor-frying after the Ming Dynasty.⁷⁾ In the Ming Dynasty, liquor-frying referred to the soaking of rhubarb in liquor before frying; however, it now refers to spraying rhubarb with liquor before frying. We have also elucidated that the anthraquinone content of modern liquor-fried rhubarb is higher than that of the rhubarb produced in ancient times.¹⁰⁾ Thus, the medicinal effects of liquor-fried rhubarb may have changed.

With respect to the production of JD, in ancient times, there were two methods, steaming and steaming after processing with liquor (liquor-steaming); however, at present, JD refers to liquor-steamed rhubarb, again supporting the notion that the medicinal effects of JD may have changed. However, the differences in the medicinal effects of the two processed rhubarbs have not been elucidated, and the method of liquor-steaming is linking to liquor-soaking or liquor-frying is also unclear.

In this report, we searched the Chinese medical literature published since the Jin and Yuan Dynasties to elucidate the medicinal effect of steamed (St) rhubarb and liquor-steamed rhubarb. Next, we prepared St rhubarb and two types of liquor-steamed rhubarb, rhubarb sprayed with 16% ethanol (as a substitute for *huangjiu*) before being steamed (LSp + St; now defined as JD) and rhubarb soaked in 16% ethanol before being steamed (LSO + St) and compared their principal compound contents; i.e., those of sennoside A, sennoside B, aloemodin, rhein, emodin, chrysophanol, physcion, lindleyin, isolindleyin, and total tannin, to elucidate the differences between steamed rhubarb and liquor-steamed rhubarb.

Medicinal effect of steamed rhubarb and liquor-steamed rhubarb according to the Chinese medicinal literature published since the Jin and Yuan Dynasties

We searched two pieces of Chinese medical literature published since the Jin and Yuan Dynasties, the first described a type of medicine named *Honzo-syo* in Japanese (*Bencao-shu* in Chinese), and the other described the production of Kampo medicines and is known as the *Iho-syo* in Japanese (*Yifang-shu* in Chinese).

We mainly found descriptions of liquor-steamed

rhubarb in the *Iho-syo*. The *Iho-syo* was written by doctors; therefore, we think that liquor-steamed rhubarb was used by doctors, and we found that the medicinal effects of steamed rhubarb were used as a remedy for poison, improving blood stasis, and moistening the bowel, while there were no descriptions of the effects of liquor-steamed rhubarb against poison (Table 1).

Table 1 The medicinal effects of steamed rhubarb and liquor-steamed rhubarb according to Chinese medical literature

(a) Medical literature describing the medicines known as *Honzo-syo* in Japanese (*Bencao-shu* in Chinese).

Steaming			
Medicinal effects Dynasty	Remedy for a poison	Improving blood stasis	Moistening the bowel
Ming	○ ^{11b-d)}		○ ^{11a)}
Qing	○ ^{11e)}		○ ^{11e)}

Liquor-steaming			
Medicinal effects Dynasty	Remedy for a poison	Improving blood stasis	Moistening the bowel
Qing			○ ^{11f,11g)}

(b) Medical literature describing the production of Kampo medicines, which is referred to as the *Iho-syo* in Japanese (*Yifang-shu* in Chinese).

Steaming			
Medicinal effects Dynasty	Remedy for a poison	Improving blood stasis	Moistening the bowel
Ming		○ ^{12a)}	○ ¹³⁾
Qing	○ ¹⁵⁾		

Liquor-steaming			
Medicinal effects Dynasty	Remedy for a poison	Improving blood stasis	Moistening the bowel
Jin and Yuan		○ ^{12b)}	
Ming			○ ^{13,14)}
Qing		○ ¹⁶⁾	○ ¹⁶⁾

Comparison of principal compound contents in steamed rhubarb and liquor-steamed rhubarb

Material and methods

Crude drug material: We used three kinds of rhubarb. 1: Rhubarb purchased from Tochimoto Tenkaido Co., Ltd. (Lot 007008001, 2008)

2: Sinsyu-daiou purchased from Tochimoto Tenkaido Co., Ltd. (Lot 007007003, 2008)

3: Gaou purchased from Uchida Wakanyaku Co., Ltd. (Lot 74T0301, 2008)

Reagents: Sennoside A and sennoside B were purchased from Wako Pure Chemical Industries, Ltd.; aloe-emodin, rhein, emodin, and chrysophanol were from Funakoshi, Ltd.; physcion and gallic acid were from Nakalai Tesque, Ltd.; Folin-Ciocalteu's reagent was from Merck, Ltd.; and lindleyin and isolindleyin were provided by Tsumura Co., Ltd. All chemicals were of an analytical grade, and the chromatographic solvents were of an HPLC grade.

Preparation of steamed rhubarb: Five grams of rhubarb chips were used in all examinations. The approximate number of chips of rhubarb 1, 2, and 3 were 690, 1000, and 173 /5 g, respectively.

We used two methods to prepare the steamed rhubarb.

(1) Steaming rhubarb in a pot

Rhubarb chips were wrapped with paper and steamed in a pot for 60 to 240 minutes. Then, the chips were dried overnight in an oven set to 40°C.

(2) Steaming rhubarb by autoclave treatment

Rhubarb chips were placed into a culture bottle, and the top was blocked with paper. Then, they were steamed by autoclave (SD-30N, Tomy Seiko Co., Ltd., Tokyo, Japan) treatment (120°C, 2 atm) for 20 to 40 minutes, and the chips were dried overnight in an oven set to 40°C.

Rhubarb dried overnight in an oven was used as a control.

Preparation of liquor-steamed rhubarb: We used two processes to produce liquor-steamed rhubarb (liquor-spraying rhubarb before steaming it and liquor-soaking rhubarb before steaming it). Sixteen % ethanol was used as a substitute for *huangjiu* (Chinese fermented wine). We previously found that rhubarb processed with ethanol showed hardly any differences from that processed with *huangjiu*.¹⁷⁾

(1) Liquor-spraying of rhubarb before it had been steamed (LSp + St)

Sixteen % ethanol was sprayed onto rhubarb chips six

times (approximate amount was 4 mL) and left for an hour. The chips were then steamed in an autoclave for 20 minutes as in the method used to process the steamed rhubarb.

(2) Liquor-soaking of rhubarb before it had been steamed (LSo + St)

Rhubarb chips were soaked in 40 mL of 16% ethanol and stirred to ensure thorough saturation with alcohol, left for 24 hours, and removed from the alcohol, and then the excess fluid was absorbed with paper. Then, the chips were steamed in an autoclave for 20 minutes as in the method used to process the steamed rhubarb.

Analysis of principal compound contents: We used an HPLC method to determine sennoside A, sennoside B, anthraquinones, lindleyin and isolindleyin contents, and used a Folin-Ciocalteu method to determine total tannin content. The HPLC method and Folin-Ciocalteu method were performed as described in a previous paper.¹⁷⁾

Statistical analysis: We performed all examination used 3 kinds of rhubarb, and obtained value from them were averaged. The significance of differences was determined by the *t*-test. All data are expressed as mean \pm S.D.

Results

Henceforth, we refer to sennoside A and sennoside B; 5 kinds of anthraquinone (aloe-emodin, rhein, emodin, chrysophanol, and physcion); and lindleyin and isolindleyin as sennoside, anthraquinone, and lindleyin, respectively.

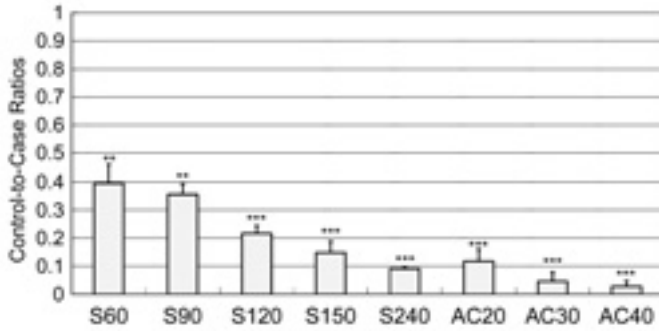
Since three kinds of rhubarb denoted the same tendency, we used averaged value.

1. Changes in the contents of the principal compounds in the steamed rhubarb

The sennoside content decreased significantly as steaming time increased. The steamed rhubarb using an autoclave for 20 minutes had an intermediate sennoside content compared to the rhubarb steamed in the pot for 150 to 240 minutes (Fig. 1). On the other hand, the anthraquinone and lindleyin contents of rhubarb were hardly changed by steaming (Fig. 2, Fig. 3), while its

total tannin content was decreased slightly compared with that of the control (Fig. 4).

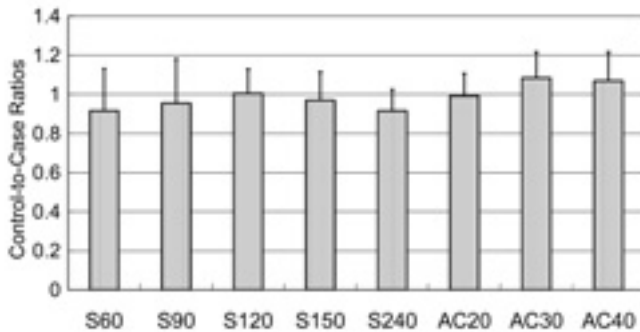
Henceforth, the rhubarb steamed in an autoclave for 20 minutes is referred to as steamed rhubarb.



S60 - 240: Steamed for 60min to 240min, AC20 - 40: Subjected to autoclave treatment for 20min to 40min

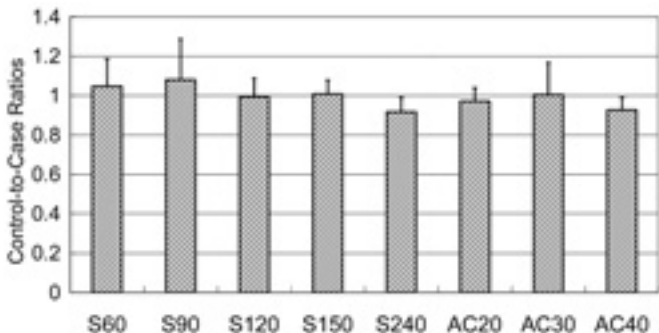
** p < 0.01, *** p < 0.005 compared with control

Fig. 1 Sennoside A and sennoside B content in the steamed rhubarb (n = 3)



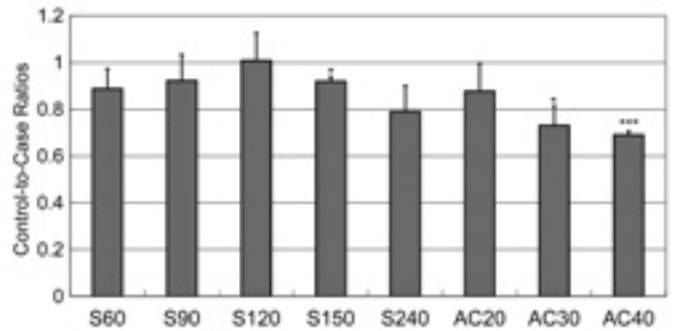
S60 - 240: Steamed for 60min to 240min, AC20 - 40: Subjected to autoclave treatment for 20min to 40min

Fig. 2 Anthraquinone content in the steamed rhubarb (n = 3)



S60 - 240: Steamed for 60min to 240min, AC20 - 40: Subjected to autoclave treatment for 20min to 40min

Fig. 3 Lindleyin and isolindleyin content in the steamed rhubarb (n = 3)



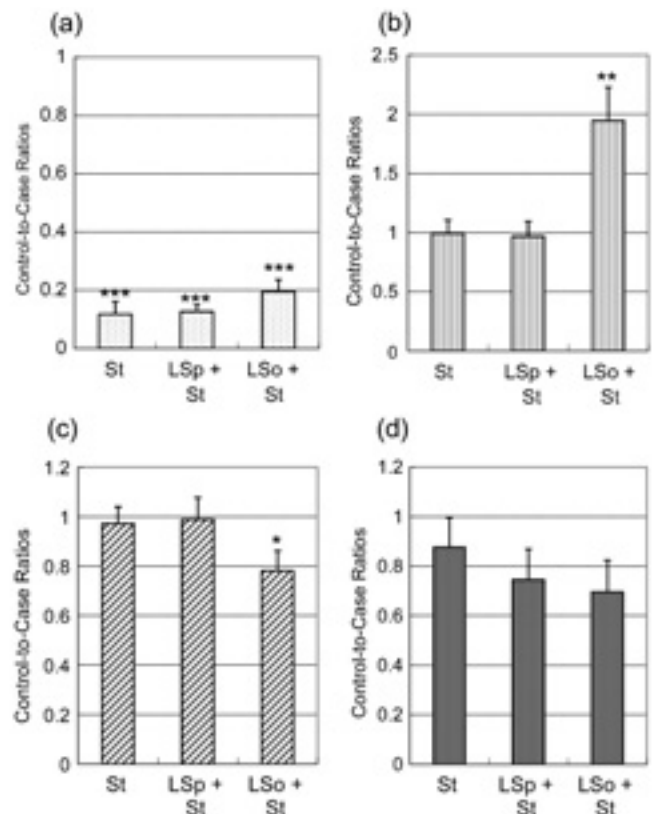
S60 - 240: Steamed for 60min to 240min, AC20 - 40: Subjected to autoclave treatment for 20min to 40min

* p < 0.05, *** p < 0.005 compared with the control

Fig. 4 Total tannin content in the steamed rhubarb (n = 3)

2. Comparison of principal compound contents in St, LSp + St, and LSo + St rhubarb

The sennoside contents in the St, LSp + St, and LSo + St were decreased significantly by 20% of the control value (Fig. 5-a).



* p < 0.05, ** p < 0.01, *** p < 0.005 compared with control

Fig. 5 Comparison of principal compound contents in steamed (St) rhubarb, rhubarb that was liquor-sprayed before being steamed (LSp + St), and rhubarb that was liquor-soaked before being steamed (LSo + St) (n = 3)

(a) Sennoside A and sennoside B, (b) Anthraquinone, (c) Lindleyin and isolindleyin, and (d) Total tannin

The anthraquinone contents in the St and LSp + St were the same as in the control. However, that in the LSo + St was increased significantly by twofold of the control value (Fig. 5-b).

The lindleyin contents in the St and LSp + St were same as that in the control. However, that in the LSo + St was decreased significantly by 80% of the control value (Fig. 5-c).

The total tannin contents in the LSp + St and LSo + St were decreased by 80% or less of the control value and were lower than that in the St (Fig. 5-d).

Discussion

We elucidated the differences between steamed rhubarb and liquor-steamed rhubarb as follows:

1. The effects of steaming

We found that steaming was the only method that drastically decreased the sennoside content and that the decrease was greater as the steaming time increased. We previously found that soaking and heating decreased the content of dianthrone compounds; i.e., sennosides, and increased that of mono-anthrone compounds; i.e., anthraquinones.^{8,10} However, steaming only decreased the content of dianthrone compounds. We think that steaming flexes the cell, causing it to elute water-soluble compounds.

2. The effects of liquor-steaming

We investigated two types of liquor-steamed rhubarb. One was LSo + St rhubarb, which had an increased anthraquinone content and a decreased sennoside content. The other was LSp + St rhubarb, which has a similar composition to St rhubarb.

Liquor-soaking increases the anthraquinone content and decreases the sennoside content⁸ while steaming decreases the sennoside content. Therefore, we suggest that LSo + St rhubarb has a lower sennoside content than liquor-soaked rhubarb and that rhubarb also has a strong anthraquinone effect.

We previously elucidated that liquor-sprayed rhubarb does not show changes in the contents of its principal compounds.¹⁰ Therefore, the composition of LSp + St is the same as that of St.

3. Medicinal effects of steamed rhubarb and liquor-steamed rhubarb

We also investigated the medicinal effects of steamed rhubarb and liquor-steamed rhubarb described in the Chinese medical literature published since the Jin and Yuan Dynasties. The former was used as a remedy for poison, improving blood stasis, and moistening the bowel, while the latter was used for improving blood stasis and moistening the bowel. Furthermore, our experiment confirmed these medicinal effects.

(1) Poison remedy

We found that steamed rhubarb was used as a remedy against poison in ancient times. However, in this experiment, the content of anthraquinones, which have antibacterial³ and anti-inflammatory⁴ effects linked to poison remedies, was increased significantly only in the LSo + St rhubarb. It is doubtful that liquor-steamed rhubarb was not used as a remedy for poison in ancient times, LSo + St rhubarb might not have been used either.

(2) Improving blood stasis

Both steamed rhubarb and liquor-steamed rhubarb were used to improve blood stasis in ancient times.

In this experiment, we found that all of the St, LSo + St, and LSp + St had drastically decreased sennoside contents. However, the contents of lindleyin and tannin were almost unchanged or slightly decreased, and only the anthraquinone content of LSp + St rhubarb was increased. Therefore, we think that all processed rhubarb has a relatively strong anti-inflammatory effect due to the presence of anthraquinones⁴ and lindleyins.⁵ It may also be effective as a remedy for blood stasis,^{8,17} and we think that the effect of LSo + St rhubarb is the strongest. These results agree with the descriptions in the ancient Chinese medicinal literature.

(3) Moistening the bowel

In ancient times, both steamed rhubarb and liquor-steamed rhubarb were used to moisten the bowel.

The purgative compounds in rhubarb are sennoside.^{1,2} The sennoside contents of all processed rhubarbs were drastically decreased. Therefore, we think that sennosides have a purgative effect by stimulating the bowel; however, this is different from moistening the bowel, which we could not confirm as an effect of processed rhubarb.

4. The effect of *Jyuku-daiou* (JD)

At present, JD refers to LSp + St rhubarb. Our results show that the effect of LSp + St is the same as that of St, which may be one of the reasons that JD is now used to refer to liquor-steamed rhubarb. However, we have not been able to elucidate the merit of liquor spraying rhubarb.

Through this study, we performed herbological and chemical studies; however pharmacological study is required to reveal activity of processed rhubarb.

Conclusion

We elucidated the differences between steamed rhubarb and liquor-steamed rhubarb. Steaming is the method that decreases only the sennoside content. While liquor-steaming of LSo + St increases its anthraquinone content and drastically decreases its sennoside content, LSp + St is now used to produce JD and has the same effects as St.

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