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Studies on Toad Venom (3): Effect of Metals on the Quality of Toad Venom Torrefied on a Metal Plate

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To study the quality of toad venom dried on different metal plates by heating at 105°C, each 20 g sample of fresh toad venom collected in Hei-Long-Jiang Province, China, was dried on (1) brass, (2) copper, (3) glass, (4) acrylic resins, (5) aluminum and (6) stainless-steel, respectively. Twelve bufadienolides, including bufalin and bufotalin, in each sample were then quantitatively analyzed by HPLC. The total levels of bufadienolides in 1000.0 mg of the dried samples were (1) > (2) > (3) > (4) > (5) > (6), varying from 303.44 mg to 420.72 mg. Besides, the color of dried venom became darker in the order of (2), (4), (6), (3), (1) and (5). Though (1) was not in good color, it was superior to the others in chemical quality. These results suggest that it is possible to dry toad venom in short period by heating it at a high temperature on a tray made of brass. This will be a better method for making high quality toad venom than the traditional method. Moreover, the removal of impurities in the fresh venom by the process of filtration through silk succeeded in raising the bufadienolides content significantly.

Key words——toad venom; bufadienolides; quality; HPLC; metal

INTRODUCTION

As previously published, it is possible to dry toad venom by heating it at a high temperature for a short period, and this method can remarkably shorten the term for making high quality toad venom, in comparison to the traditional processing method in China that needed more than two years for drying venom.[1] We visited Shang-Dong Province and Hei-Long-Jiang Province, the biggest and secondary biggest origin of fresh toad venom in China, respectively, from 1997 to 2000. In the traditional processing method, no metal except aluminum was used during the production process,[2] and steel was generally avoided in the manufacturing process because steel has been thought to change the color of fresh venom dark and reduce the quality. In this report, to examine the effect of metals on the quality of toad venom, fresh toad venom collected in Hei-Long-Jiang Province was torrefied on different metal plates such as brass, copper, aluminum, stainless steel, glass, and acrylic resins, then twelve bufadienolides including bufalin and bufotalin in each sample were quantitatively analyzed using reversed-phase HPLC. Moreover, the collecting method for fresh toad venom was improved.

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MATERIALS AND METHODS

Fresh Toad Venom Fresh toad venom was collected in Hei-Long-Jiang Province, China, in 2000, by traditional method, nipping the secretion gland with a specialized tool made by aluminum as previously published.[1] Subsequently, the fresh toad venom was filtrated through silk to remove foreign matter.

Drying Method Each 20 g sample of fresh toad venom was made into thick disks of about 25 mm I.D.×5 mm height by hand, and placed on a different plates, as (1) brass, (2) copper, (3) glass, (4) acrylic resins, (5) aluminum, and (6) stainless steel. And then dried at 105°C in an electric oven. The sample on each plate was checked for consistency every 20 minutes by using fingers to press down on the sample in order that the ending point be known. The samples were then placed into the desiccator with silica gel to cool. In cases that two successive samples produced the same results, the drying process was considered finished. At the same time, a 20 g sample of fresh toad venom was freeze-dried to constitute the control.

Assay of Twelve Bufadienolides

Standards Bufalin (BL), cinobufagin (CB), and resibufogenin (RB) were obtained from Yoneyama Industrial Co., Ltd. Arenobufagin (ARB), bufotalin (BT), cinobufotalin (CBT), desacetyl-

Table 1. Bufadienolides Contents of Toad Venom by Using Various Material for Drying

	GBT	ABG	HBG	DACG	DACT	DABL	BT	MB	CBT	BL	CBG	RBG	TOTAL
fresh	22.80±0.79	22.46±0.78	trace	0.96±0.03	1.29±0.04	7.97±0.28	11.86±0.41	trace	4.21±0.15	74.07±2.57	154.49±5.35	149.53±5.18	450.55±15.61
brass	27.93±1.48	20.73±1.10	trace	0.88±0.05	1.02±0.05	7.31±0.39	12.90±0.68	trace	3.87±0.20	67.85±3.59	141.84±7.51	136.39±7.22	420.72±22.26
copper	27.36±1.19	20.29±0.88	trace	0.83±0.04	0.88±0.04	6.78±0.03	12.56±0.55	trace	3.71±0.16	65.51±2.86	134.71±5.87	130.62±5.69	403.25±17.58
glass	19.00±1.62	19.31±1.65	trace	0.86±0.07	0.96±0.08	6.51±0.56	11.83±1.01	trace	3.50±0.30	62.20±5.31	127.51±10.89	122.94±10.50	374.62±32.01
acrylic resins	18.84±2.04	17.86±1.93	trace	0.81±0.09	1.04±0.11	6.64±0.72	11.46±1.24	trace	3.41±0.37	60.99±6.66	126.05±13.63	125.77±13.60	372.87±40.33
aluminum	17.76±2.05	18.33±2.11	trace	0.86±0.10	0.98±0.11	7.03±0.81	11.57±1.33	trace	3.42±0.39	59.36±6.85	126.32±14.57	122.74±14.16	368.37±42.48
stainless steel	15.98±1.52	15.28±1.46	trace	0.57±0.05	1.37±0.13	0.00±0.00	0.73±0.07	trace	3.00±0.29	52.16±4.98	109.79±10.47	104.56±9.97	303.44±28.95

Each value shows mean±S.D. of mg/1000.0 mg dehydrated sample.

bufotalin (DABT), desacetylcinobufagin (DACG), desacetylcinobufotalin (DACT), gamabufotalin (GBT), hellebrigenin (HBG), and marinobufagin (MB) were provided from Professor Shimada, Kanazawa university.[1,3]

Analyzing Method, HPLC Conditions, and Color Value All the analyzing methods and conditions in analyzing by HPLC and colorimeter are the same as reported in the previous paper.[1,4] Additionally, after each sample was powdered, all the materials were dried again at 105°C in an electric oven for 5 hours (to obtain the loss on drying values to enable revision of the contents ratios).

RESULTS

1. The total bufadienolides levels in 1000.0 mg of each dehydrated venom sample were as follows (n=3); 450.55 mg±1.56 mg as freeze-dried fresh venom; 420.72 mg±2.23 mg from brass; 403.25 mg±1.76 mg from copper; 374.62 mg±3.20 mg from glass; 372.87 mg±4.03 mg from acrylic resins; 368.37 mg±4.25 mg from aluminum; and 303.44 mg±2.89 mg from stainless steel, respectively. The results show that the level of bufadienolides greatly differs from each other depending on the plate material used, and that brass is the best metal to make high quality toad venom while steel is worst (Table 1).

2. The color value of L^* of toad venom was as follows (n=5); 45.96±0.97 for brass; 41.23±0.70 for copper; 45.96±0.68 for glass; 44.72±0.47 for acrylic resins; 47.47±1.10 for aluminum; and 45.75±2.22 for stainless steel, respectively. The results show that the color of powdered toad venom had no relation to the contents of bufadienolides (Table 2).

3. There were no significant differences in the ratio of each bufadienolide in each sample dried on the plate of different materials, except for the venom that was dried on stainless steel. It contained few DABT and BT, compared with the other samples.

Table 2. Color of Powdered Toad Venom Manufacturd by Using Different Materials

	n	L^*	a^*	b^*
brass	5	45.96±0.97	4.48±0.47	18.93±0.82
copper	5	41.23±0.70	6.78±0.28	18.71±0.91
glass	5	45.96±0.68	5.67±0.30	21.64±0.83
acrylic resins	5	44.72±0.47	6.76±0.09	20.34±0.54
alminum	5	47.47±1.10	5.24±0.14	18.93±0.82
stainless steel	5	46.15±0.68	5.33±0.44	19.12±2.24

$L^*a^*b^*$ color system: L^* indicate lightness from dark to light with numerical values 0 to 100. a^* and b^* indicate hue with $+a^*$ the red, $-a^*$ the green, $+b^*$ the yellow and $-b^*$ the blue, and larger absolute value shows more vivid and strong in each color.
Each value represent mean±S.D., respectively.

4. No significant differences in the length of time for drying each sample on the different plates were recognized: 220 minutes for brass; 240 minutes for copper; 260 minutes for glass; 260 minutes for acrylic resins; 260 minutes for aluminum; and 280 minutes for stainless steel, respectively.

DISCUSSION

1. It was clarified that fresh toad venom could be dried on a hot brass plate to make high quality toad venom in a short time period, while stainless steel should not be used for the same way of drying. The level of bufadienolides in dried venom on a hot stainless plate was about 30% less than in venom dried on a brass plate.

2. As previously reported, meat-colored toad venom is considered to be the highest quality, in the toad venom production center. No metal except aluminum is used in the process of making toad venom. In China in the past, the quality of toad venom might decrease due to the use of steel in the drying process, so they might stop using metals. But brass and copper were found through this study to be better materials than glass or acrylic resin for drying venom in an oven.

3. As it rusts easily, iron may not be a suitable metal for use in the process of drying watery toad venom. From this point of view, stainless steel may be ideal. However, bufadienolide levels in toad venom dried on a stainless steel plate showed obviously less amounts than that dried on brass. Therefore, it can be said that iron should not be used during the process of drying the venom.

4. Thermal conduction (cal/cm/sec/deg) of the metals and other materials used in this study was as follows; 0.26 for brass; 0.94 for copper; 0.64 to 0.73 for glass, 0.55 for aluminum; and 0.04 to 0.06 for stainless steel, respectively.[5] The values show that there is no relationship between thermal conduction and the level of bufadienolides. Therefore, the difference in the levels of bufadienolides depends on another factors.

5. In a previous report, it was noted that the contents of bufadienolides should decrease while drying at low temperatures, such as 60℃.[1] At this time, the drying temperature was constant and there were no significant differences in the lengths of time for drying each sample on the different plates. However, there were minor differences between the different plates in terms of the lengths of time for drying. The shortest time was observed with brass, and the longest time with stainless steel. At high temperatures, in order to maintain the high contents of bufadienolides, the length of time for drying should be strictly controlled. This is because bufadienolides are easily destroyed at high temperatures, such as 105℃.

6. At this time, the fresh toad venom was immediately filtrated through silk. As a result, the contents of bufadienolides were significantly enhanced, compared with the results obtained in the previous report.[1] There may be some factors behind this result. For example, the raw materials were not the same, the producing regions were not the same, etc. However, the elimination of foreign matter, such as skin, muscle, rubbish, etc., must be considered the principal reason behind this result. To manufacture good quality toad venom, the fresh toad venom must be filtrated through silk to omit foreign matter.

7. There are three types of toad venom on the Chinese market.[6] One is the same as on Japanese markets. The second, called ''Coin Type Toad Venom'', is made in Shanghai, and is smaller and darker than the former. The third, called ''Plate Type Toad Venom'', is made in Shang-Dong and An-Hui Provinces, and looks like a thin plate like a glass' slide. In the past, all of these three types of toad venom were imported to Japan. The new method of manufacturing toad venom is thought to be a good way of making Plate Type Toad Venom.

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