

Investigating Storage Mode and Shelf Life of Bagged TCM Decoctions under Different Conditions

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Abstract [Objectives] To investigate optimal storage methods and shelf life determination for several representative bagged traditional Chinese medicine (TCM) decoctions under centralized preparation conditions in intelligent TCM pharmacies. [Methods] First, the nourishing formula was prepared and packaged in bags. Under the three storage conditions of 37 °C before cold storage (including full high temperature), cold storage before 37 °C (including full cold storage), and alternating 37 °C and cold storage, the 30 d cycle was investigated to determine the total microbial colony count, so as to determine a reasonable storage method of traditional Chinese medicine decoction. Secondly, five representative prescriptions were prepared and packaged in bags, stored under 37 °C, room temperature and cold conditions. The investigation period was 30 d. The pH, total bacterial count and soluble solid content were measured, and the changes of each index were analyzed to obtain the shelf life of the bagged Chinese medicine decoction. [Results] First, the nourishing formula was investigated for 30 d. The microbial results of refrigeration after 1–2 d of 37 °C, complete refrigeration and 37 °C cooling with an alternate interval of 2 d or less met the requirements, while the microbial results of refrigeration after 3 d or above of 37 °C, refrigeration after 1–5 d and then 37 °C, complete 37 °C, 37 °C and cooling with an alternate interval of 3 d or above excessive microorganism. Second, under the condition of 37 °C storage, the pH of the five prescriptions decreased significantly, the total microbial colonies exceeded the standard, and the solid content decreased significantly. However, under the condition of room temperature and cold storage, the pH, total microbial colonies, and solid content of the five prescriptions remained stable. [Conclusions] The first is to refrigerate the decoction after 1–2 d of 37 °C, completely refrigerate it, and refrigerate the decoction with an alternate interval of 2 d or less at 37 °C. The shelf life can last for 30 d. Several storage conditions are conducive to guiding the development of the storage mode of the decoction. Second, under the conditions of cold storage, all the indexes were stable, and the shelf life of the five representative formulas was 30 d.

Key words Bagged TCM decoctions, Shelf-life determination, Total microorganism, pH, Solid content

1 Introduction

Intelligent traditional Chinese medicine pharmacy is a comprehensive use of Internet, IoT, cloud computing, big data of technologies, combined with automatic control technology, through connecting with medical institutions to obtain electronic prescriptions, provide full process pharmaceutical services including professional prescription review, Chinese medicine preparation, decoction, packaging and delivery.

As one of the traditional Chinese medicine formulas, traditional Chinese medicine decoction has been the main dosage form for clinical use due to its flexible formulation, dialectical applicability, simple preparation, and rapid onset of effect. With the gradual increase of China's aging population, the number of people taking traditional Chinese medicine decoctions has been increasing year by year. Therefore, ensuring the quality of traditional Chinese medicine decoctions requires attention. How to determine the expiration date of traditional Chinese medicine decoctions has attracted the attention of consumer groups, patients, and peers. We have conducted key technology research on the production, processing, storage, transportation, and quality and safety control of various bagged traditional Chinese medicine, aiming to improve the level of pharmaceutical hygiene and safety, ensure the stability of product manufacturing, standardize and optimize production

processes, and improve product quality.

2 Materials and methods

2.1 Equipment YJD20B-G ten-function automatic decoction machine; YB50-250 Traditional Chinese Medicine Soup Packaging Machine; DNP-9162BS-III electric constant temperature incubator; SW-CJ-2FDG double person single side vertical clean workbench; BYC-310 Medical Refrigerator; HK-3C desktop precision acidity meter; Sartorius BS 224S electronic analytical balance.

2.2 Microbial medium and preparation Nutrient agar culture medium; preparation made from self-made bagged boiled solution of traditional Chinese medicine.

2.3 Supplier preparation

2.3.1 Preparation of nourishing formulas. The test sample was selected as a nourishing formula with easy swelling bags, and the prescription is shown in Table 1. It was used to determine the effects of changes in bacteria and pH of bagged traditional Chinese medicine under different transportation temperature environments^[1].

2.3.2 Preparation of representative prescriptions. The supply used 5 different formulas, including headache calming formula, heat clearing cold formula, lumbar disc formula, prostate formula, cough formula, etc. The formulas are shown in Table 1, used to determine the effects of changes in bacteria, pH, and solid content of different formulations under different cooling conditions, and under 37 °C, room temperature, and refrigeration conditions^[2].

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Table 1 Prescriptions of each group of formulas

Prescription type	Formulas
Nourishing Formula	20 g of <i>Atractylodes macrocephala</i> , 30 g of <i>Artemisia scoparia</i> , 20 g of stir fried sour jujube kernels, 10 g of <i>Radix Aucklandiae</i> , 20 g of <i>Poria cocos</i> (Schw.) Wolf, 30 g of Coix seed, 20 g of <i>Astragalus membranaceus</i> , 20 g of ripe <i>Codonopsis pilosula</i> , 5 g of <i>Angelica sinensis</i> , 5 g of prepared <i>Radix Polygalae</i> , 10 g of <i>Amomum tsaoko</i> Crevost & Lem, and 20 g of <i>Longan Arillus</i>
Headache Calming Formula	15 g of <i>Pinellia ternata</i> , 30 g of <i>Gastrodia elata</i> , 30 g of <i>Atractylodes macrocephala</i> , 30 g of seed of wild jujube, 30 g of <i>Ligusticum wallichii</i> , 45 g of <i>Achyranthes bidentata</i> , 45 g of <i>Paeonia lactiflora</i> , 30 g of <i>Angelica dahurica</i> , 45 g of <i>Pueraria lobata</i> , 9 g of <i>Asarum</i> , 45 g of Acacia bark, and 45 g of <i>Acorus calamus</i>
Qingre Cold Formula	30 g of <i>Trichosanthis Radix</i> , 30 g of <i>Viola philippica</i> , 30 g of <i>Radix Aucklandiae</i> , 30 g of <i>Fructus Gardeniae</i> , 30 g of <i>Radix paeoniae rubra</i> , 30 g of <i>Schizonepeta</i> , 30 g of Honeysuckle, 30 g of <i>Saponilla</i> , 30 g of Daqingye, 30 g of Dandelion, and 30 g of Cicada molting
Lumbar Intervertebral Disc Square Formula	70 g of <i>Atractylodes macrocephala</i> , 70 g of <i>Schizonepeta tenuifolia</i> , 70 g of <i>Platycodon grandiflorus</i> , 70 g of <i>Rehmannia glutinosa</i> , 63 g of roasted licorice, 70 g of <i>Astragalus membranaceus</i> , 105 g of dried tangerine peel, 70 g of mulberry parasitism, 105 g of <i>Angelica dahurica</i> , 105 g of <i>Astragalus membranaceus</i> , 105 g of dried <i>Codonopsis pilosula</i> , and 70 g of dried <i>Codonopsis pilosula</i>
Prostate Formula	30 g of cherry blossom meat, 30 g of cooked <i>Rehmannia glutinosa</i> , 30 g of yam, 30 g of thicket fruit, 45 g of <i>Radix millettiae</i> Speciosae, 30 g of raspberry, 30 g of mulberry cuttlebone, and 15 g of Semen Astragali Complanati
Cough Formula	30 g of <i>Fructus Xanthii</i> , 15 g of <i>Fritillaria thunbergii</i> Miq. , 45 g of <i>Peucedanum praeruptorum</i> Dunn, 15 g of Licorice, 45 g of Bitter Almond, 45 g of <i>Platycodon grandiflorus</i> , 69 g of <i>Morus alba</i> L. , 45 g of Loquat Leaves, 45 g of <i>Flos Magnoliae Liliflorae</i> , 45 g of <i>Forsythia suspensa</i> , 90 g of Coix seed, 45 g of <i>Saposhnikovia Radix</i> , 30 g of Mint, 60 g of winter melon seed, 90 g of <i>Houttuynia cordata</i> , and 90 g of reed stem

2.3.3 The methods of decocting soup ingredients. The decoction methods are based on the *Management Standards for Traditional Chinese Medicine Decoction Rooms in Medical Institutions*.

2.4 Experimental design methods

2.4.1 Nourishing formula. A nourishing formula was selected and the experimental soup was divided into three groups. The first group was first refrigerated in a 37 °C incubator and then refrigerated in a 4 – 6 °C refrigerator. The second group was refrigerated first and then in a 37 °C incubator. The third group was alternately refrigerated in a 37 °C incubator. The experimental period was set

at one month, and the experiment was divided into three stages based on the actual medication situation of the patient. The first stage was 3 d before storage, and it was stipulated to open one package every day to measure the chemical and microbiological indicators; the second stage was from the 3rd to the 12th day of storage, with one package to be unpacked every 3 d for testing of chemical and microbiological indicators; the third stage was from the 12th to the 30th d of storage, with one package to be unpacked every 6 days for testing of chemical and microbiological indicators^[3–4]. See Table 2 below.

Table 2 Design of microbial testing conditions

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
First high temperature followed by refrigeration																														
Full high temperature																														
First cold storage and then high temperature																														
Full of cold storage																														√
Alternating between high temperature and cold storage																														

NOTE White squares represent samples stored at 37 °C , while gray squares represent samples stored at low temperatures (4 – 6 °C) . The same below.

2.4.2 Representative prescriptions. Headache Calming Formula, Qingre Cold Formula, Lumbar Intervertebral Disc Square Formula, Prostate Formula, Cough Formula, *etc.* were selected for storage at 37 °C , room temperature (20 – 25 °C) , and refrigera-

tion (4–6 °C). Physical, chemical, and microbiological indicators such as pH, total bacterial count, color appearance, and solid content were tested at 0, 3, 7, 14, and 30 d^[5].

3 Methods and results

3.1 Test results of nourishing formula

Microbial testing was

conducted using three stages: 37 °C followed by refrigeration (including the entire process), 37 °C followed by refrigeration (including full refrigeration), and 37 °C alternating with refrigeration. The results of microbial testing are shown in Table 3, and the pH test results are shown in Table 4.

Table 3 Microbial results of nourishing formula under different conditions												cfu/mL
Days	0	1	2	3	4	5	6	9	12	18	24	30
First 37 °C, then cold storage	50	60	80	90			120	130	150	180	220	340
			80	120			180	200	200	220	260	350
				100			150	200	280	280	340	1 800
							250	298	350	468	1 680	5 900
Cold storage first and then 37 °C	80	90	24	126			302	350	420	560	1650	8870
			90	136			288	310	388	450	1520	3 720
				80			220	282	330	420	1480	2 600
							120	202	320	420	550	1 700
37 °C alternating with Cold storage	60	80					100	100	120	150	220	250
			100	150			200	250	280	350	420	538
			60	90	120		180	190	220	260	280	480
			80	120	120		200	240	250	280	320	588
		60	100	140			160	188	250	390	550	1 950

Table 4 The pH results of nourishing formulas under different conditions												
Days	0	1	2	3	4	5	6	9	12	18	24	30
First 37 °C, then Cold storage	5.06	5.06	4.99	5.09			5.06	5.06	5.05	5.05	5.03	5.02
			4.97	5.02			5.03	5.03	5.02	5.03	5.01	5.01
				4.92			5.15	5.14	5.12	5.13	5.12	4.95
							4.81	4.81	4.79	4.77	4.68	4.56
Cold storage first and then 37 °C	5.06	5.08	5.08	5.02			4.88	4.88	4.80	4.75	4.70	4.55
			5.15	5.09			4.94	4.91	4.87	4.80	4.64	4.48
				5.13			4.95	4.90	4.87	4.76	4.61	4.53
							5.10	5.01	4.98	4.90	4.81	4.62
37 °C alternating with Cold storage	5.06	5.06						5.05	5.08	5.07	5.04	5.03
			4.99	4.97			5.10	5.06	5.06	5.01	5.01	4.81
			5.08	5.08	5.12		5.06	4.98	4.81	4.83	4.80	4.72
			5.06	4.97	5.02		5.04	5.02	5.00	4.87	4.83	4.79
		5.08	5.15	5.09			5.10	5.06	5.02	4.96	4.85	4.80

3.2 Test results of different formulas By using different formulas, the samples were boiled and cooled naturally or cooled with water, and stored at 37 °C, room temperature (20–25 °C), and refrigerated (4–6 °C) for 0, 3, 7, 14, and 30 d. Physical and chemical measurements of pH, total bacterial count, and solid content were conducted. The results are shown in Table 5.

4 Conclusions and discussions

4.1 Analysis of results under three stage conditions of nourishing formula results under three stage conditions of nourishing formula By analyzing the changes in colony count and pH under different storage conditions in the analysis table, some findings are revealed.

(i) First, maintaining a constant temperature of 37 °C, then

refrigerating to a storage condition of 4 °C. Storing at 37 °C for 1 d, then refrigerating for 2 d and continuing to store at 4 °C for 30 d for sampling microbial testing. Among them, the colony count increased from 50 to 340 cfu/mL after being refrigerated at 37 °C for 1 d, and the pH decreased from 5.06 to 5.02; after being placed at high temperature for 2 d, the number of colonies refrigerated increased from 50 to 350 cfu/mL, and the pH decreased from 5.06 to 5.01; the colony count and pH meet the regulations. If placed under 37 °C conditions for 3 d or more and inspected continuously for 30 d for sampling microbial testing, the bacterial count will exceed the standard and the pH will significantly decrease. Therefore, after the preparation of bagged traditional Chinese medicine, they need to be refrigerated for a maximum duration of 2 d at 37 °C to ensure that microorganisms do not grow rapidly.

Table 5 Test results of different formulas

Prescription type	Condition	pH					Total bacterial count					Solid content				
		0 d	3 d	7 d	14 d	30 d	0 d	3 d	7 d	14 d	30 d	0 d	3 d	7 d	14 d	30 d
Headache Calming Formula	37 °C	5.06	5.11	5.12	5.09	5.08	100	120	200	600	2700	6.12	6.10	6.08	6.02	5.18
	Normal temperature		5.09	5.11	5.12	5.08		100	180	300	450		6.01	6.03	6.02	6.01
	Cold storage		5.12	5.15	5.06	5.10		100	150	160	220		6.06	6.08	6.06	6.05
Qingre Cold Formula	37 °C	5.32	5.22	5.28	5.13	4.94	180	220	340	644	5800	4.52	4.32	4.26	5.33	3.26
	Normal temperature		5.33	5.33	5.31	5.30		190	28	500	620		4.50	4.50	4.49	4.47
	Cold storage		5.37	5.35	5.36	5.35		200	220	260	300		4.45	4.66	4.49	4.50
Lumbar Intervertebral Disc Square Formula	37 °C	4.84	4.86	4.82	4.72	4.65	120	172	300	340	8000	10.98	10.45	10.25	9.24	7.14
	Normal temperature		4.91	4.85	4.8	4.83		220	252	360	520		10.77	10.85	10.64	10.35
	Cold storage		4.9	4.92	4.94	4.99		160	214	260	372		10.64	10.55	10.44	10.50
Prostate Formula	37 °C	4.57	4.53	4.49	4.55	4.52	20	60	220	350	6800	8.99	8.98	8.78	8.50	7.01
	Normal temperature		4.58	4.60	4.73	4.62		60	120	260	360		8.91	8.89	8.85	8.65
	Cold storage		4.61	4.62	4.67	4.61		70	80	120	150		8.93	8.90	8.89	8.80
Cough Formula	37 °C	5.11	5.03	4.99	5.02	5.11	50	120	250	450	9200	9.01	8.98	8.78	8.60	5.49
	Normal temperature		5.07	5.12	5.11	5.09		100	210	300	410		8.99	8.94	8.95	8.85
	Cold storage		5.09	5.07	5.08	5.08		80	90	120	190		8.99	8.96	8.90	8.90

(ii) First, refrigerating the medicinal liquid at 4 °C for 1–5 d, and then placing it under 37 °C conditions for 30 d to conduct microbiological testing. The bacterial count of the samples exceeded the standard; after being refrigerated at 4 °C for 30 d, the bacterial count increased from 50 cfu/mL to 250 cfu/mL, and the pH decreased from 5.06 to 5.03. The microorganisms and pH remained good. Therefore, we can see that the shelf life of bagged traditional Chinese medicine can be maintained for at least 30 d when kept in a refrigerator.

(iii) Alternating storage between 37 °C and 4 °C 37 °C for 1 and 2 d, and continuing to inspect and test the microbial colony count for 30 d. The results all meet the regulations; the storage conditions with a 3-day interval between high temperature of 37 °C and refrigeration temperature of 4 °C exceeded the standard after 30 d. Therefore, the storage interval for the alternating cold and hot cycles is 1–2 d, and the shelf life can be 30 d.

4.2 Test results of five formulas under different conditions

The headache calming formula, heat clearing cold formula, lumbar intervertebral disc formula, prostate formula, cough formula, *etc.* were boiled and subjected to pH, colony number, and solid content tests under 37 °C, room temperature, and refrigeration conditions. The pH did not show significant changes after continuous storage for 30 d; five formulas were stored at 37 °C, and the microbial count exceeded the limit (high temperature storage for 30 d; headache calming formula 100 cfu/mL → 2 700 cfu/mL; clearing heat and cold formula 180 cfu/mL → 5 800 cfu/mL; lumbar intervertebral disc formula 120 cfu/mL → 8 000 cfu/mL; prostate formula 20 cfu/mL → 6 800 cfu/mL; cough formula 50 cfu/mL → 9 200 cfu/mL), but both room temperature and refrigeration met the requirements; the solid content decreased significantly after 30 d high-temperature storage (high temperature storage for 30 d; headache calming formula 6.12 → 5.18; heat clearing cold formula 4.52 → 3.26; lumbar disc formula 10.98 → 7.14; prostate formula 8.99 → 7.01; cough formula 9.01 →

5.49), while the decrease was not significant during refrigeration. Therefore, under refrigerated storage conditions, the 5 formulas had the best refrigeration effect, with a shelf life of 30 d. At room temperature, considering the temperature differences in different regions, further microbial investigation is needed.

After the overall testing of different formulas mentioned above, based on our company’s existing conditions of decoction, storage and cooling, we can ensure a 30-d shelf life of traditional Chinese medicine decoctions, ensuring that customers can take the decoctions within the shelf life or a shorter period of time. In addition, we can also shorten the storage time of traditional Chinese medicine decoctions as much as possible according to the method of splitting and boiling, and ensure timely delivery to customers within the transportation time. It is recommended to refrigerate and store the decoctions as soon as possible to ensure their quality.

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